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FILE 'USPAT' ENTERED AT 08:36:35 ON 18 FEB 97
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*           W E L C O M E   T O   T H E
*           U . S .   P A T E N T   T E X T   F I L E
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=> s tgf/
'TGF/' IS NOT A VALID FIELD CODE

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=> s tgf?
L1          974 TGF?

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=> e tgf
E#      FILE          FREQUENCY  TERM
--      ---          -
E1      USPAT          10      TGEXCH/BI
E2      USPAT           1      TGEZ/BI
E3      USPAT          920 --> TGF/BI
E4      USPAT           1      TGF0/BI
E5      USPAT           5      TGF1/BI
E6      USPAT           7      TGF2/BI
E7      USPAT           2      TGF200001/BI
E8      USPAT           1      TGF4/BI
E9      USPAT           1      TGF5/BI
E10     USPAT          13      TGFA/BI
E11     USPAT           2      TGFA2/BI
E12     USPAT           2      TGFA3/BI

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=> s e3
L2          920 TGF/BI

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=> e tgfb
E#      FILE          FREQUENCY  TERM
--      ---          -
E1      USPAT           3      TGFALPHA/BI
E2      USPAT           1      TGFAPE40/BI
E3      USPAT          46 --> TGFB/BI
E4      USPAT           2      TGFB1/BI
E5      USPAT           1      TGFB2/BI
E6      USPAT           1      TGFB3/BI
E7      USPAT           2      TGFBETA/BI
E8      USPAT           1      TGFB5/BI
E9      USPAT           1      TGFECEB/BI
E10     USPAT           2      TGFET/BI
E11     USPAT           1      TGFFEG/BI
E12     USPAT           2      TGFI/BI

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=> s e3,e4,e5,e6,e7
      46 TGFB/BI
      2 TGFB1/BI
      1 TGFB2/BI
      1 TGFB3/BI
      2 TGFBETA/BI
L3      51 (TGFB/BI OR TGFB1/BI OR TGFB2/BI OR TGFB3/BI OR TGFBETA/BI)

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=> s l2 or l3
L4      950 L2 OR L3
=> s l4 and extracell?(2w)matri?

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      5972 EXTRACELL?
      135479 MATRI?
      995 EXTRACELL?(2W)MATRI?
L5      148 L4 AND EXTRACELL?(2W)MATRI?

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=> s 15 and antibody?  
22176 ANTIBOD?  
L6 83 L5 AND ANTIBOD?  
=> D L6 1-20 CIT,AB

1. 5,602,156, Feb. 11, 1997, Method for inhibiting metalloproteinase expression; Elise C. Kohn, et al., 514/359, 255, 256, 258, 261, 383, 396, 398, 400 [IMAGE AVAILABLE]

US PAT NO: 5,602,156 [IMAGE AVAILABLE] L6: 1 of 83

ABSTRACT:

Calcium homeostasis is an important regulator of MMP-2 transcription, activation and activity. Disclosed herein are compounds which inhibit the expression of matrix metalloproteinases in cells. Pharmaceutical application of these compounds to inhibit the expression of MMPs offers a new approach to cancer treatment as well as treatment for nerve healing, degenerative cartilaginous diseases, decubitus ulcers, arthritis, Alzheimer's disease, wound healing, proliferative retinopathy, proliferative renal diseases, corneal ulcers and fertility problems.

2. 5,599,788, Feb. 4, 1997, Method for accelerating skin wound healing with H3 protein; Anthony F. Purchio, et al., 514/2; 424/278.1, 409; 514/12, 885, 886, 887, 944, 945, 946, 947 [IMAGE AVAILABLE]

US PAT NO: 5,599,788 [IMAGE AVAILABLE] L6: 2 of 83

ABSTRACT:

A method of producing recombinant transforming growth factor .beta.-induced H3 protein and the use of this protein to accelerate wound healing. H3 promoted adhesion of human dermal fibroblasts to tissue culture plastic. The protein is applied directly to a wound or is used to promote adhesion and spreading of dermal fibroblasts to a solid support such as a nylon mesh which is then applied to the wound. In addition, CHO cells expressing H3 inhibited tumor cell growth.

3. 5,596,072, Jan. 21, 1997, Method of refolding human IL-13; Janice Culpepper, et al., 530/351; 424/85.2; 435/69.1; 530/402, 412; 930/141 [IMAGE AVAILABLE]

US PAT NO: 5,596,072 [IMAGE AVAILABLE] L6: 3 of 83

ABSTRACT:

Nucleic acids encoding human IL-13, and purified IL-13 proteins and fragments thereof. \*\*Antibodies\*\*, both polyclonal and monoclonal, are also provided. Methods of using the compositions for both diagnostic and therapeutic utilities are provided.

4. 5,595,885, Jan. 21, 1997, Matrix metalloproteinase inhibitor peptides; William G. Stetler-Stevenson, et al., 435/69.2, 172.3, 252.3; 536/23.1, 23.5, 25.3 [IMAGE AVAILABLE]

US PAT NO: 5,595,885 [IMAGE AVAILABLE] L6: 4 of 83

ABSTRACT:

The present invention is an isolated protein of 21,600 Da which binds to both latent and activated type IV collagenase with high affinity at 1:1 molar stoichiometry, thereby abolishing enzyme activity. The protein is

purified by affinity chromatography on solid phase metalloproteinase, or solid phase metalloproteinase substrates which bind the enzyme-inhibitor complex. The complete primary structure of this protein (initially called CSC-21K), as determined by sequencing overlapping peptides spanning the entire protein, reveals homology with a protein called TIMP, Tissue Inhibitor of Metalloproteinases. In addition, a cDNA for this novel inhibitor, now designated TIMP-2, was cloned from a melanoma cell and its sequence was compared with that of human TIMP-1. Northern blots of melanoma cell mRNA showed two distinct transcripts of 0.9 kb and 3.5 kb which are down-regulated by transforming growth factor- $\beta$ , and are unchanged by phorbol ester treatment. The inhibitor of the present invention may be used for treatment of pathologic conditions resulting from inappropriate degradation of **extracellular** **matrix** molecules by matrix metalloproteinases, such as metastatic neoplasia, myocardial infarction, and arthritis. Therapeutic treatments using this inhibitor may include formulations for inhalation and inclusion complexes adapted for buccal or sublingual administration, or administration of a recombinant DNA molecule which expresses a DNA segment that encodes the matrix metalloproteinase inhibitor of this invention.

5. 5,595,722, Jan. 21, 1997, Method for identifying an agent which increases **TGF**- $\beta$  levels; David J. Grainger, et al., 424/9.2 [IMAGE AVAILABLE]

US PAT NO: 5,595,722 [IMAGE AVAILABLE]

L6: 5 of 83

ABSTRACT:

A method for identifying a compound that is a **TGF**- $\beta$  activator or production stimulator is provided.

6. 5,594,120, Jan. 14, 1997, Integrin alpha subunit; Michael B. Brenner, et al., 536/23.5; 435/172.3, 240.2, 320.1; 536/24.31, 24.33 [IMAGE AVAILABLE]

US PAT NO: 5,594,120 [IMAGE AVAILABLE]

L6: 6 of 83

ABSTRACT:

The present invention relates to a novel integrin  $\alpha$ .sup.E subunit and its functional equivalents. The invention further includes pharmaceutical compositions containing the isolated peptides, oligonucleotides encoding the peptides, vectors containing the oligonucleotides, and cell lines transfected with the vectors.

7. 5,591,716, Jan. 7, 1997, Beneficial wound healing applications of calreticulin and other hyaluronan-associated proteins; John W. Siebert, et al., 514/12; 530/350, 395, 399 [IMAGE AVAILABLE]

US PAT NO: 5,591,716 [IMAGE AVAILABLE]

L6: 7 of 83

ABSTRACT:

Hyaluronan associated proteins, in particular calreticulin, promote the accelerated and relatively scarless healing of wounds. Methods for treating wounds using such proteins, and pharmaceutical compositions comprising such proteins, are provided.

8. 5,585,087, Dec. 17, 1996, Assay for identifying extracellular signaling proteins; Kevin D. Lustig, et al., 424/9.2, 9.1, 93.1, 93.2; 435/4, 6, 7.21, 7.4, 69.1, 172.3, 240.2, 320.1 [IMAGE AVAILABLE]

US PAT NO: 5,585,087 [IMAGE AVAILABLE]

L6: 8 of 83

ABSTRACT:

The present invention concerns a novel paracrine signaling assay.

9. 5,583,103, Dec. 10, 1996, Inhibition of transforming growth factor beta activity; Erkki I. Ruoslahti, et al., 514/8, 2; 530/350, 380, 399 [IMAGE AVAILABLE]

US PAT NO: 5,583,103 [IMAGE AVAILABLE]

L6: 9 of 83

ABSTRACT:

The present invention provides a method of inhibiting an activity of \*\*TGF\*\*.beta. comprising contacting the \*\*TGF\*\*.beta. with a purified decorin. In a specific embodiment, the present invention relates to the ability of decorin, a 40,000 dalton protein that usually carries a glycosaminoglycan chain, to bind \*\*TGF\*\*.beta.. The invention also provides a novel cell regulatory factor designated MRF. Also provided are methods of identifying, detecting and purifying cell regulatory factors and proteins which bind and affect the activity of cell regulatory factors.

10. 5,580,979, Dec. 3, 1996, Phosphotyrosine peptidomimetics for inhibiting SH2 domain interactions; William W. Bachovchin, 540/509, 505, 510, 511, 542, 569, 570, 571, 572 [IMAGE AVAILABLE]

US PAT NO: 5,580,979 [IMAGE AVAILABLE]

L6: 10 of 83

ABSTRACT:

The present invention makes available novel compounds represented by the general formula ##STR1## wherein Y represents a phosphate analog. Which compounds are useful for inhibiting an interaction between a protein containing an SH2 domain and a phosphotyrosine-containing polypeptide.

11. 5,580,756, Dec. 3, 1996, B7IG fusion protein; Peter S. Linsley, et al., 435/69.7, 91.1; 530/350, 387.1, 387.3, 395; 536/23.4 [IMAGE AVAILABLE]

US PAT NO: 5,580,756 [IMAGE AVAILABLE]

L6: 11 of 83

ABSTRACT:

The invention identifies the B7 antigen as a ligand that is reactive with the CD28 receptor on T cells. Fragments and derivatives of the B7 antigen and CD28 receptor, including fusion proteins having amino acid sequences corresponding to the extracellular domains of B7 or CD28 joined to amino acid sequences encoding portions of human immunoglobulin C.gamma.1, are described. Methods are provided for using B7 antigen, its fragments and derivatives, and the CD28 receptor, its fragments and derivatives, as well as \*\*antibodies\*\* and other molecules reactive with B7 antigen and/or the CD28 receptor, to regulate CD28 positive T cell responses, and immune responses mediated by T cells. The invention also includes an assay method for detecting ligands reactive with cellular receptors mediating intercellular adhesion.

12. 5,580,722, Dec. 3, 1996, Methods of determining chemicals that modulate transcriptionally expression of genes associated with cardiovascular disease; J. Gordon Foulkes, et al., 435/6, 91.1, 91.2;



US PAT NO: 5,580,722 [IMAGE AVAILABLE]

L6: 12 of 83

ABSTRACT:

The invention provided for a method of directly and specifically transcriptionally modulating the expression of a gene encoding a protein of interest associated with treatment of one or more symptoms of a cardiovascular disease such as atherosclerosis, restenosis or hypertension.

Further provided is a method of determining whether a molecule not previously known to be a modulator of protein biosynthesis is capable of directly and specifically transcriptionally modulating the expression of a gene encoding a protein of interest associated with treatment of one or more symptoms of a cardiovascular disease.

Lastly, the invention provides a method of directly and specifically transcriptionally modulating in a human being the expression of a gene encoding a protein of interest associated with treatment of one or more symptoms of a cardiovascular disease, thus ameliorating the disease.

13. 5,578,703, Nov. 26, 1996, Substantially pure receptor like TGF- $\beta$ 1 binding molecules; Hidenori Ichijo, et al., 530/350, 395 [IMAGE AVAILABLE]

US PAT NO: 5,578,703 [IMAGE AVAILABLE]

L6: 13 of 83

ABSTRACT:

The invention relates to a family of substantially pure, receptor like \*\*TGF $\beta$ 1 binding glycoproteins. These molecules are characterized by molecular masses of 160 kd, 70-80 kd, and 30-40 kd as determined by SDS-PAGE, and the ability to bind the \*\*TGF $\beta$ 1 molecule. This family of molecules is useful in identifying and/or quantifying \*\*TGF $\beta$ 1 in a sample, as well as inhibiting its effect on cells. Also described are nucleic acid sequences which code for the protein monomer making up the molecules.

14. 5,571,893, Nov. 5, 1996, Cardiac hypertrophy factor; Joffre Baker, et al., 530/350, 351, 399; 930/140 [IMAGE AVAILABLE]

US PAT NO: 5,571,893 [IMAGE AVAILABLE]

L6: 14 of 83

ABSTRACT:

Isolated CHF, isolated DNA encoding CHF, and recombinant or synthetic methods of preparing CHF are disclosed. These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

15. 5,571,675, Nov. 5, 1996, Detection and amplification of cadiotrophin-1(cardiac hypertrophy factor); Joffre Baker, et al., 435/6, 91.2, 91.21; 536/24.3, 24.31, 24.32, 24.33 [IMAGE AVAILABLE]

US PAT NO: 5,571,675 [IMAGE AVAILABLE]

L6: 15 of 83

ABSTRACT:

Isolated CHF, isolated DNA encoding CHF, and recombinant or synthetic

methods of preparing CHF are disclosed. These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

16. 5,567,807, Oct. 22, 1996, Processes for the purification of human recombinant decorin and the detection of guanidinium ions; William S. Craig, et al., 530/395, 412, 416 [IMAGE AVAILABLE]

US PAT NO: 5,567,807 [IMAGE AVAILABLE]

L6: 16 of 83

ABSTRACT:

The present invention is directed to a process for the production of substantially pure human recombinant decorin which involves the combination of three separate stages characterized by contacting decorin-containing cell culture medium with (1) a first strong anionic exchange resin; then with (2) a hydrophobic interactive chromatographic resin; and finally with (3) a second strong anionic exchange resin. By using a combination of steps, and certain reagents, in particular a 2.4 to 3 molar GuHCl solution to elute decorin from a hydrophobic interactive column, the process of this invention provides a more convenient and reproducible process for purifying human recombinant decorin. The invention also provides a process for detecting the presence of guanidinium ions in a sample solution. The detection process involves contacting a sample solution suspected of containing guanidinium ions with a cation exchange resin and eluting the guanidinium ions present in tire sample solution with an aqueous buffer solution having a pH of about 1.5 to about 2. This is followed by contacting the eluant with a cation suppressor columns and simultaneously flowing a suppressor regenerate solution in the opposite direction on the opposite side of the permeable membrane of the column, and finally, detecting the presence of guanidinium ions in the eluant from the ion exchange column which was contacted with the suppressor column by use of a conductivity detector.

17. 5,567,609, Oct. 22, 1996, Use of isolated domains of type IV collagen to modify cell and tissue interactions; Michael P. Sarras, Jr., et al., 435/240.2; 530/356 [IMAGE AVAILABLE]

US PAT NO: 5,567,609 [IMAGE AVAILABLE]

L6: 17 of 83

ABSTRACT:

The instant invention demonstrates that the 7S and NC1 domains of type IV collagen disrupts cell aggregation and tissue development. Structural changes in mesoglea, inhibition of cell proliferation, and changes in cell differentiation patterns accompanies the blockage of cell aggregates which indicate that blockage may be due to alterations in mesoglea (\*\*extracellular\*\* \*\*matrix\*\*) structure with accompanying effects on cell behavior. Type IV collagen has a critical role in the initial formation of mesoglea and that perturbation of mesoglea formation affects cell division, cell differentiation, and morphogenesis.

18. 5,567,584, Oct. 22, 1996, Methods of using biologically active dimerized polypeptide fusions to detect PDGF; Andrzej Z. Sledziewski, et al., 435/6, 7.1, 69.7; 436/501; 536/23.4 [IMAGE AVAILABLE]

US PAT NO: 5,567,584 [IMAGE AVAILABLE]

L6: 18 of 83

ABSTRACT:

Methods for producing secreted receptor analogs and biologically active peptide dimers are disclosed. The methods for producing secreted receptor analogs and biologically active peptide dimers utilize a DNA sequence encoding a receptor analog or a peptide requiring dimerization for biological activity joined to a dimerizing protein. The receptor analog includes a ligand-binding domain. Polypeptides comprising essentially the extracellular domain of a human PDGF receptor fused to dimerizing proteins, the portion being capable of binding human PDGF or an isoform thereof, are also disclosed. The polypeptides may be used within methods for determining the presence of and for purifying human PDGF or isoforms thereof.

19. 5,563,146, Oct. 8, 1996, Method of treating hyperproliferative vascular disease; Randall E. Morris, et al., 514/291; 424/122 [IMAGE AVAILABLE]

US PAT NO: 5,563,146 [IMAGE AVAILABLE]

L6: 19 of 83

ABSTRACT:

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal by administering an antiproliferative effective amount of rapamycin alone or in combination with mycophenolic acid.

20. 5,563,124, Oct. 8, 1996, Osteogenic product and process; Christopher J. Damien, et al., 514/21; 623/16, 17, 22 [IMAGE AVAILABLE]

US PAT NO: 5,563,124 [IMAGE AVAILABLE]

L6: 20 of 83

ABSTRACT:

Disclosed is a product which includes calcium carbonate and bone growth factor useful for the promotion of bone formation when implanted in the body. The calcium carbonate is preferably in the form of aragonite which can be recovered from naturally occurring coral. A preferred bone growth factor of the present invention is a protein mixture purified from bone. Also disclosed is a process for the induction of bone formation which includes implanting the product in a body. The product and process of the present invention are particularly useful in hip replacement operations, knee replacement operations, spinal fusion operations, repair of periodontal defects, treatment of osteoporosis, repair of bone tumor defects and repair of bone fractures.

=> D L6 21-40 CIT,AB

21. 5,559,022, Sep. 24, 1996, Liver reserve cells; Brian A. Naughton, et al., 435/240.2; 424/93.1; 435/240.1 [IMAGE AVAILABLE]

US PAT NO: 5,559,022 [IMAGE AVAILABLE]

L6: 21 of 83

ABSTRACT:

The present invention relates to liver reserve or progenitor cells. In particular, it relates to the isolation, characterization, culturing, and uses of liver reserve cells. Liver reserve cells isolated by density gradient centrifugation can be distinguished from other liver parenchymal cells by their morphology, staining characteristics, high proliferative activity and ability to differentiate in vitro. In long-term cultures described herein, these cells expand in numbers and differentiate into

morphologically mature liver parenchymal cells, capable of mediating liver-specific functions. Therefore, isolated liver reserve cells may have a wide range of applications, including, but not limited to, their uses as vehicles of exogenous genes in gene therapy, and/or to replace and reconstitute a destroyed, infected, or genetically deficient mammalian liver by transplantation.

22. 5,549,674, Aug. 27, 1996, Methods and compositions of a bioartificial kidney suitable for use in vivo or ex vivo; H. David Humes, et al., 623/11; 435/240.2, 240.21; 514/12; 600/36; 604/6, 35, 48, 319; 623/1, 12 [IMAGE AVAILABLE]

US PAT NO: 5,549,674 [IMAGE AVAILABLE]

L6: 22 of 83

ABSTRACT:

A novel cell seeded hollow fiber bioreactor is described as a potential bioartificial kidney. Renal cells are seeded along a hollow fiber in a perfused bioreactor to reproduce the ultrafiltration function and transport function of the kidney. Maintenance of tissue specific function and ultrastructure suggest that this bioreactor provides an economical device for treating renal failure as well as studying renal tubululogenesis in vitro.

23. 5,545,569, Aug. 13, 1996, Prevention and treatment of pathologies associated with abnormally proliferative smooth muscle cells; David J. Grainger, et al., 436/518 [IMAGE AVAILABLE]

US PAT NO: 5,545,569 [IMAGE AVAILABLE]

L6: 23 of 83

ABSTRACT:

\*\*TGF\*\*-beta activators and \*\*TGF\*\*-beta production stimulators are employed to maintain or increase vessel lumen diameter in a diseased or injured vessel of a mammal. Conditions such as restenosis following angioplasty, vascular bypass grafts, transplanted organs, atherosclerosis or hypertension are characterized by a reduced vessel lumen diameter. In a preferred embodiment of the invention, \*\*TGF\*\*-beta activators and production stimulators inhibit abnormal proliferation of smooth muscle cells. \*\*TGF\*\*-beta activators or production stimulators that are not characterized by an undesirable systemic toxicity profile at a prophylactic dose are also amenable to chronic use for prophylactic purposes with respect to disease states involving proliferation and/or migration of vascular smooth muscle cells over time. Further provided is a method for determining \*\*TGF\*\*-beta in vitro, thereby identifying a patient at risk for atherosclerosis and monitoring a recipient that has received one or more administrations of a \*\*TGF\*\*-beta activator or production stimulator.

24. 5,543,394, Aug. 6, 1996, Bone morphogenetic protein 5 (BMP-5) compositions; John M. Wozney, et al., 514/12; 424/484; 435/69.1; 530/350, 395, 399 [IMAGE AVAILABLE]

US PAT NO: 5,543,394 [IMAGE AVAILABLE]

L6: 24 of 83

ABSTRACT:

Purified BMP-5 proteins and processes for producing them are disclosed. The proteins may be used in the treatment of bone and/or cartilage defects and in wound healing and related tissue repair.

25. 5,538,892, Jul. 23, 1996, Nucleic acids encoding a \*\*TGF\*\*-.beta. type 1 receptor; Patricia K. Donahoe, et al., 435/240.2, 69.1, 252.3, 254.11, 320.1; 536/23.5, 24.31 [IMAGE AVAILABLE]

US PAT NO: 5,538,892 [IMAGE AVAILABLE]

L6: 25 of 83

ABSTRACT:

Isolated DNAs (e.g., cDNAs or genomic fragments) encoding \*\*TGF\*\*-.beta. type I receptors, or soluble, ligand-binding fragments thereof; vectors or cells which contain such DNAs; and substantially pure polypeptides encoded by such DNAs, whether produced by expression of the isolated DNAs, by isolation from natural sources, or by chemical synthesis.

26. 5,538,866, Jul. 23, 1996, Prostate-specific membrane antigen; Ron S Israeli, et al., 435/69.3, 240.2, 252.3, 254.2, 320.1; 536/23.5; 935/12, 23, 27, 66 [IMAGE AVAILABLE]

US PAT NO: 5,538,866 [IMAGE AVAILABLE]

L6: 26 of 83

ABSTRACT:

This invention provides an isolated mammalian nucleic acid molecule encoding a mammalian prostate-specific membrane antigen. This invention also provides prostate-specific membrane nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid molecule encoding a mammalian prostate-specific membrane antigen. This invention further provides vector and vector host expression system for the prostate-specific membrane antigen. This invention also provides methods to identify ligands which bind to the prostate-specific membrane antigen, to generate \*\*antibody\*\* against a complete prostate-specific membrane antigen or a portion of the antigen. This invention further provides purified prostate-specific membrane antigen. This invention provides a therapeutic agent comprising an \*\*antibody\*\* directed against to prostate-specific membrane antigen and a cytotoxic agent conjugated thereto. This invention also provides a method of imaging prostate cancer and an immunoassay for measuring the amount of the prostate-specific membrane antigen in a biological sample. This invention further provides transgenic nonhuman mammal which comprises the isolated nucleic acid molecule encoding a mammalian prostate-specific membrane antigen.

27. 5,534,615, Jul. 9, 1996, Cardiac hypertrophy factor and uses therefor; Joffre Baker, et al., 530/350; 424/569, 570; 530/380 [IMAGE AVAILABLE]

US PAT NO: 5,534,615 [IMAGE AVAILABLE]

L6: 27 of 83

ABSTRACT:

Isolated CHF, isolated DNA encoding CHF, and recombinant or synthetic methods of preparing CHF are disclosed. These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

28. 5,532,156, Jul. 2, 1996, Hepatocyte cell line derived from the epiblast of pig blastocysts; Neil Talbot, et al., 435/240.2, 240.21, 240.23, 240.243 [IMAGE AVAILABLE]

US PAT NO: 5,532,156 [IMAGE AVAILABLE]

L6: 28 of 83

ABSTRACT:

Continuous cultures of pluripotent parenchymal hepatocytes were derived from the epiblasts of pig blastocysts. The cultures are feeder-dependent and grow slowly with doubling times of 3 to 4 days. They differentiate into large secretory duct-like structures or form small canaliculi. Alternatively, the cells accumulate droplets that stain intensely with oil red O, a lipid-specific stain.  $\alpha$ -Fetoprotein and albumin mRNA expression increases as the cells differentiate in culture.

29. 5,532,132, Jul. 2, 1996, Method for regulating formation of a complex of plasminogen activator, its receptor and inhibitor; Ning Wang, et al., 435/7.21, 7.23, 7.9; 436/501, 503 [IMAGE AVAILABLE]

US PAT NO: 5,532,132 [IMAGE AVAILABLE]

L6: 29 of 83

ABSTRACT:

The present invention is directed to a method for regulating formation of a complex of a plasminogen activator, its receptor and one of its inhibitors. More specifically, this method involves contacting a target cell having a plasminogen activator receptor with a compound which interacts with a component of the complex such that a change in target cell cytoskeletal stiffness results.

30. 5,521,288, May 28, 1996, CD28IG fusion protein; Peter S. Linsley, et al., 530/387.3; 435/7.2, 7.92, 69.1, 69.7, 91.1, 240.1, 252.3, 252.33, 320.1; 530/300, 350, 387.1, 395, 409, 866, 867, 868; 536/23.1, 23.4, 23.53 [IMAGE AVAILABLE]

US PAT NO: 5,521,288 [IMAGE AVAILABLE]

L6: 30 of 83

ABSTRACT:

The invention identifies the B7 antigen as a ligand that is reactive with the CD28 receptor on T cells. Fragments and derivatives of the B7 antigen and CD28 receptor, including fusion proteins having amino acid sequences corresponding to the extracellular domains of B7 or CD28 joined to amino acid sequences encoding portions of human immunoglobulin C.gamma.1, are described. Methods are provided for using B7 antigen, its fragments and derivatives, and the CD28 receptor, its fragments and derivatives, as well as **antibodies** and other molecules reactive with B7 antigen and/or the CD28 receptor, to regulate CD28 positive T cell responses, and immune responses mediated by T cells. The invention also includes an assay method for detecting ligands reactive with cellular receptors mediating intercellular adhesion.

31. 5,520,926, May 28, 1996, Method of using mannose phosphates for the treatment of fibrotic disorders; Mark W. J. Ferguson, 424/443, 422, 444, 445, 446, 447, 448, 449; 514/23 [IMAGE AVAILABLE]

US PAT NO: 5,520,926 [IMAGE AVAILABLE]

L6: 31 of 83

ABSTRACT:

Mannose-6- and 1-phosphates and their pharmaceutically acceptable salts and bioprecursors thereof are useful in the treatment of fibrotic disorders. They accelerate wound healing and the 6-phosphate prevents or mitigates scar formation. The invention includes particular, appropriate

formulations of the mannose phosphate.

32. 5,516,781, May 14, 1996, Method of treating restenosis with rapamycin; Randall E. Morris, et al., 514/291 [IMAGE AVAILABLE]

US PAT NO: 5,516,781 [IMAGE AVAILABLE]

L6: 32 of 83

ABSTRACT:

This invention provides a method of preventing or treating hyperproliferative vascular disease in a mammal by administering an antiproliferative effective amount of rapamycin alone or in combination with mycophenolic acid.

33. 5,510,459, Apr. 23, 1996, Glucagon antagonists; Robert A. Smith, et al., 530/308, 324 [IMAGE AVAILABLE]

US PAT NO: 5,510,459 [IMAGE AVAILABLE]

L6: 33 of 83

ABSTRACT:

Methods for detecting glucagon antagonists through the use of recombinant DNA techniques are provided. Briefly, subsequent to the expression of glucagon analogs within suitable host cells, the analogs are exposed to a glucagon receptor coupled to a response pathway in the presence of native glucagon. A reduction in the stimulation of the response pathway resulting from the binding of the glucagon analog to the glucagon receptor relative to the stimulation of the response pathway by native glucagon alone indicates the presence of a glucagon antagonist. Glucagon antagonists identified and isolated through the methods are also provided.

34. 5,510,328, Apr. 23, 1996, Compositions that inhibit wound contraction and methods of using same; James Polarek, et al., 514/8, 14, 16, 17; 530/326 [IMAGE AVAILABLE]

US PAT NO: 5,510,328 [IMAGE AVAILABLE]

L6: 34 of 83

ABSTRACT:

The present invention provides methods for reducing or inhibiting wound contraction in a subject having a wound comprising administering to the subject a pharmaceutical composition comprising a peptide or a polypeptide. The invention provides, for example, a method of reducing or inhibiting wound contraction comprising the administration of a pharmaceutical composition comprising a peptide having more than three consecutive basic amino acids. The invention also provides a method of reducing or inhibiting wound contraction comprising the administration of a pharmaceutical composition comprising decorin.

35. 5,508,188, Apr. 16, 1996, Method of growing cells in a mammal; Sanford H. Barsky, et al., 435/240.2, 240.1, 240.25 [IMAGE AVAILABLE]

US PAT NO: 5,508,188 [IMAGE AVAILABLE]

L6: 35 of 83

ABSTRACT:

Human basement membrane matrix is provided, produced by a novel tumorigenic cell line, where the basement membrane can be used for the growth of a variety of cells, in culture and in vivo. Other cell lines are provided, which may serve to evaluate in vivo the response of tumorigenic cells to various agents, including basement membrane. The

basement membrane finds use in allowing the growth of cells in culture and in vivo, particularly cells which are otherwise refractory to xenografting.

36. 5,486,599, Jan. 23, 1996, Construction and use of synthetic constructs encoding syndecan; Scott Saunders, et al., 530/395; 435/69.1, 69.7, 252.3, 320.1; 536/23.4, 23.5; 935/10, 47, 50, 70 [IMAGE AVAILABLE]

US PAT NO: 5,486,599 [IMAGE AVAILABLE]

L6: 36 of 83

ABSTRACT:

A purified mammalian proteoglycan, and genetic information encoding such proteoglycans, having a core polypeptide molecular weight of about 30 kD to about 35 kD, and comprising a hydrophilic amino terminal extracellular region, a hydrophilic carboxy terminal cytoplasmic region, a transmembrane hydrophobic region between said cytoplasmic and extracellular regions, a protease susceptible cleavage sequence extracellularly adjacent the transmembrane region of the peptide, and at least one glycosylation site for attachment of a heparan sulfate chain to said extracellular region, said glycosylation site comprising a heparan sulfate attachment sequence represented by a formula Xac-Z-Ser-Gly-Ser-Gly, where Xac represents an amino acid residue having an acidic sidechain, and Z represents from 1 to 10 amino acid residues. Additional peptides having this glycosylation site and genetic information useful for preparing a number of variations based on this glycosylation site are also provided.

37. 5,484,726, Jan. 16, 1996, \*\*Antibodies\*\* specific for human stromelysin-3 and a method for detection of stromelysin-3; Paul Basset, et al., 435/7.4; 530/387.1, 387.7, 388.1, 388.26, 388.8 [IMAGE AVAILABLE]

US PAT NO: 5,484,726 [IMAGE AVAILABLE]

L6: 37 of 83

ABSTRACT:

The present invention relates to a gene encoding stromelysin-3, which is a new member of the metalloproteinase family. Expression of the stromelysin-3 gene has been found to be specifically associated with invasive breast, head, neck and skin cancer. The invention also relates to \*\*antibodies\*\* which specifically bind to human stromelysin-3 and the use of these stromelysin-3 \*\*antibodies\*\* for detection of the stromelysin-3 protein in a sample.

38. 5,476,922, Dec. 19, 1995, Methods and compositions for the preparation and use of autocrine growth factors; F. Gary Toback, et al., 530/399; 424/545, 558, 574; 530/300, 324, 414; 930/120 [IMAGE AVAILABLE]

US PAT NO: 5,476,922 [IMAGE AVAILABLE]

L6: 38 of 83

ABSTRACT:

Autocrine growth factors and isoforms of those factors have been identified, isolated, purified and manipulated. Nucleic acid segments coding for the factors, and \*\*antibodies\*\* directed to the factors are also aspects of the present invention. The effect of these growth factors on cells is to enhance their growth by increasing mitogenesis. In particular, the growth factors stimulate kidney epithelial cell growth. The growth factors differ from others previously reported in their molecular weights and other properties, for example, resistance to



denaturation by dithiothreitol. Methods of preparation and use of the factors are also described. The growth factors are released from kidney epithelial cells by short exposures to a low-sodium environment. The factors have potential for treatment of kidney disease.

39. 5,468,787, Nov. 21, 1995, Biomaterials for tissue repair; Michael Braden, et al., 523/113; 128/898; 424/78.32, 422; 523/115, 116; 524/531; 604/51, 56 [IMAGE AVAILABLE]

US PAT NO: 5,468,787 [IMAGE AVAILABLE]

L6: 39 of 83

ABSTRACT:

A curable composition of a monomeric heterocyclic acrylic or methacrylic ester and an acrylate or methacrylate polymer is used to promote tissue repair, especially cartilage repair.

40. 5,461,033, Oct. 24, 1995, Modulation of class II antigen expression; Anne Donnet, et al., 514/12; 424/85.1; 514/2, 21; 530/351 [IMAGE AVAILABLE]

US PAT NO: 5,461,033 [IMAGE AVAILABLE]

L6: 40 of 83

ABSTRACT:

The expression of Class II antigen expression by intestinal epithelial cells is modulated by administering to a mammal in need thereof an effective amount of \*\*TGF\*\*-.beta.2.

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U.S. Patent & Trademark Office LOGOFF AT 08:55:43 ON 18 FEB 97

1/3/114 (Item 113 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 2000 The Dialog Corp. All rts. reserv.

02586358

Utility  
MONOCLONAL ANTIBODIES WHICH BIND BOTH TRANSFORMING GROWTH FACTORS .BETA.1  
AND .BETA.2 AND METHODS OF USE  
[Treatment of acute liver injury]

PATENT NO.: 5,571,714  
ISSUED: November 05, 1996 (19961105)  
INVENTOR(s): Dasch, James R., Palo Alto, CA (California), US (United States  
of America)  
Pace, III, Doran R., San Francisco, CA (California), US  
(United States of America)  
Waegell, Wendy O., Mountain View, CA (California), US (United  
States of America)  
ASSIGNEE(s): Celtrix Pharmaceuticals, Inc, (A U.S. Company or Corporation),  
Santa Clara, CA (California), US (United States of America)  
[Assignee Code(s): 28382]  
APPL. NO.: 7-759,109  
FILED: September 06, 1991 (19910906)

This application is a continuation of application Ser. No. 07-288,432,  
filed Dec. 12, 1988, abandoned.

02598776

Utility

INHIBITION OF TRANSFORMING GROWTH FACTOR BETA ACTIVITY

[Binding with decorin]

PATENT NO.: 5,583,103

ISSUED: December 10, 1996 (19961210)

INVENTOR(s): Ruoslahti, Erkki I., Rancho Santa Fe, CA (California), US  
(United States of America)  
Yamaguchi, Yu, San Diego, CA (California), US (United States  
of America)

ASSIGNEE(s): La Jolla Cancer Research Foundation, (A U.S. Company or  
Corporation), La Jolla, CA (California), US (United States of  
America)  
[Assignee Code(s): 9506]

APPL. NO.: 8-212,311

FILED: March 14, 1994 (19940314)

This application is a continuation of application Ser. No. 08-050,762, filed Apr. 20, 1993, now abandoned, which is a continuation of application Ser. No. 07-467,888, filed on Jan. 22, 1990, now abandoned, which is a continuation of application Ser. No. 07-212,702, filed Jun. 28, 1988 now abandoned.

This invention was made with support of government grants CA 30199, CA 42507 and CA 28896 from the National Cancer Institute. Therefore, the United States government may have rights in the invention.

02675128

Utility

USE OF FIBROMODULIN TO PREVENT OR REDUCE DERMAL SCARRING

PATENT NO.: 5,654,270

ISSUED: August 05, 1997 (19970805)

INVENTOR(s): Ruoslahti, Erkki I., Rancho Santa Fe, CA (California), US  
(United States of America)  
Longaker, Michael T., San Francisco, CA (California), US  
(United States of America)  
Whitby, David J., Adel, GB (United Kingdom)

ASSIGNEE(s): La Jolla Cancer Research Foundation, (A U.S. Company or  
Corporation), La Jolla, CA (California), US (United States of  
America)  
[Assignee Code(s): 9506]

EXTRA INFO: Assignment transaction [Reassigned], recorded February 17,  
1998 (19980217)

APPL. NO.: 8-303,238

FILED: September 08, 1994 (19940908)

This application is a continuation of application Ser. No. 07-978,931, filed Nov. 17, 1992, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-882,345, filed May 13, 1992, now abandoned, which is a continuation of U.S. Ser. No. 07-792,192, filed Nov. 14, 1991, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-467,888, filed Jan. 22, 1990, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-212,702, filed Jun. 28, 1988, now abandoned.

1/3/93 (Item 92 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 2000 The Dialog Corp. All rts. reserv.

02732758

Utility

DECORIN FRAGMENTS INHIBITING CELL REGULATORY FACTORS

PATENT NO.: 5,705,609

ISSUED: January 06, 1998 (19980106)

INVENTOR(s): Ruoslahti, Erkki I., Rancho Santa Fe, CA (California), US  
(United States of America)  
Pierschbacher, Michael D., San Diego, CA (California), US  
(United States of America)  
Cardenas, Jose, San Diego, CA (California), US (United States  
of America)  
Craig, William, San Diego, CA (California), US (United States  
of America)  
Mullen, Daniel G., San Diego, CA (California), US (United  
States of America)

ASSIGNEE(s): La Jolla Cancer Research Foundation, (A U.S. Company or  
Corporation), La Jolla, CA (California), US (United States of  
America)  
[Assignee Code(s): 9506]

APPL. NO.: 8-442,063

FILED: May 16, 1995 (19950516)

This application is a continuation of application Ser. No. 07-865,652,  
filed Apr. 3, 1992, now abandoned which is a continuation-in-part of  
application Ser. No. 07-792,192, filed Nov. 14, 1991, now abandoned which  
is a continuation-in-part of Ser. No. 07-467,888, filed Jan. 22, 1990, now  
abandoned which is a continuation-in-part of Ser. No. 07-212,702, filed

1/3/79 (Item 78 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 2000 The Dialog Corp. All rts. reserv.

02806025

Utility

MONOCLONAL ANTIBODIES TO TRANSFORMING GROWTH FACTOR-BETA AND METHODS OF USE  
[ Neutralization of the inhibitory effects of transforming growth factor and  
hybridomas]

PATENT NO.: 5,772,998  
ISSUED: June 30, 1998 (19980630)  
INVENTOR(s): Dasch, James R., Palo Alto, CA (California), US (United States  
of America)  
Pace, III, Doran R., San Francisco, CA (California), US  
(United States of America)  
Waegell, Wendy O., Mountain View, CA (California), US (United  
States of America)  
ASSIGNEE(s): Celtrix Pharmaceuticals, Inc , (A U.S. Company or Corporation)  
, Santa Clara, CA (California), US (United States of America)  
[Assignee Code(s): 28382]  
APPL. NO.: 8-434,976  
FILED: May 04, 1995 (19950504)

This application is a division of application Ser. No. 07-759,109, filed  
Sep. 6, 1991, now U.S. Pat. No. 5,571,714, which is a continuation of

1/3/76 (Item 75 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 2000 The Dialog Corp. All rts. reserv.

02816448

Utility

MONOCLONAL ANTIBODIES TO TRANSFORMING GROWTH FACTOR-BETA AND METHODS OF USE

PATENT NO.: 5,783,185  
ISSUED: July 21, 1998 (19980721)  
INVENTOR(s): Dasch, James R., Palo Alto, CA (California), US (United States  
of America)  
Pace, III, Doran R., San Francisco, CA (California), US  
(United States of America)  
Waegell, Wendy O., Mountain View, CA (California), US (United  
States of America)  
ASSIGNEE(s): Celtrix Pharmaceuticals, Inc , (A U.S. Company or Corporation)  
, Santa Clara, CA (California), US (United States of America)  
[Assignee Code(s): 28382]  
APPL. NO.: 8-434,977  
FILED: May 04, 1995 (19950504)

This application is a division of application Ser. No. 07-759,109, filed  
Sep. 6, 1991, now U.S. Pat. No. 5,571,714, which is a continuation of  
application Ser. No. 07-288,432, filed Dec. 22, 1988, now abandoned.

1/3/9 (Item 8 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 2000 The Dialog Corp. All rts. reserv.

03154923

Utility  
MONOCLONAL ANTIBODIES TO TRANSFORMING GROWTH FACTOR-.BETA. AND METHODS OF  
USE

PATENT NO.: 6,090,383  
ISSUED: July 18, 2000 (20000718)  
INVENTOR(s): Dasch, James R., 3181 Morris Dr., Palo Alto, CA (California),  
US (United States of America), 94303  
Pace, III, Doran R., 310 Stanyan St., #102, San Francisco, CA  
(California), US (United States of America), 94118  
Waegell, Wendy O., 2019 Leghorn, Mountain View, CA  
(California), US (United States of America), 94043  
[Assignee Code(s): 68000]  
APPL. NO.: 9-97,843  
FILED: June 15, 1998 (19980615)

This application is a continuation of U.S. Ser. No. 08-434,976, filed May  
4, 1995, now U.S. Pat. No. 5,772,998, which is a division of U.S. Ser. No.  
07-759,109, filed Sep. 6, 1991, now U.S. Pat. No. 5,571,714, which is a



(Item 135 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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01963243

Utility  
METHOD OF TREATING INFLAMMATION  
[Polypeptide with amino acid sequence of cartilage inducing factor A or B]

PATENT NO.: 5,008,240  
ISSUED: April 16, 1991 (19910416)  
INVENTOR(s): Bentz, Hanne, Palo Alto, CA (California), US (United States of America)  
Ellingsworth, Larry, San Jose, CA (California), US (United States of America)  
Armstrong, Rosa, Palo Alto, CA (California), US (United States of America)  
ASSIGNEE(s): Collagen Corporation, (A U.S. Company or Corporation ), Palo Alto, CA (California), US (United States of America)  
[Assignee Code(s): 18638]  
EXTRA INFO: Assignment transaction [Reassigned], recorded August 21, 1995 (19950821)  
APPL. NO.: 7-230,330  
FILED: August 09, 1988 (19880809)  
DISCLAIMER: February 21, 2006 (20060221)

#### CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. patent application Ser. No. 836,672 filed Mar. 6, 1986, now U.S. Pat. No. 4,806,523, which is a continuation-in-part of U.S. patent application Ser. No. 763,337, filed Aug. 6, 1985, now abandoned.

FULL TEXT: 779 lines

1/3/137 (Item 136 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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01922780

Utility  
METHOD OF TREATING INFLAMMATION WITH CARTILAGE INDUCING FACTOR  
[POLYPEPTIDES, CARDIOVASCULAR DISORDERS]

PATENT NO.: 4,971,952  
ISSUED: November 20, 1990 (19901120)  
INVENTOR(s): Bentz, Hanne, Newark, CA (California), US (United States of America)  
Ellingsworth, Larry, San Jose, CA (California), US (United States of America)  
Armstrong, Rosa, Palo Alto, CA (California), US (United States of America)  
ASSIGNEE(s): Collagen Corporation, (A U.S. Company or Corporation ), Palo Alto, CA (California), US (United States of America)  
[Assignee Code(s): 18638]  
EXTRA INFO: Assignment transaction [Reassigned], recorded April 1, 1991 (19910401)  
Assignment transaction [Reassigned], recorded August 21, 1995 (19950821)

APPL. NO.: 7-263,635  
FILED: October 27, 1988 (19881027)

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of copending U.S. patent application Ser. No. 836,672 filed 6 Mar. 1986, now U.S. Pat. No. 4,806,523, which is a continuation-in-part of U.S. patent application Ser. No. 763,337, filed 6 Aug. 1985, now abandoned.

FULL TEXT: 972 lines

?

PLEASE ENTER A COMMAND OR BE LOGGED OFF IN 5 MINUTES

? t sl/kwic/136,137 .

1/KWIC/136 (Item 135 from file: 654)  
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...Implant-associated inflammation was negligible compared with the control implants. A dense collagenous connective tissue **matrix** was evident throughout the implant. Morphologically, the fibroblasts appeared to be metabolically less active than at earlier time points.

These histological observations demonstrate that CIF **inhibits** inflammatory cell function in vivo. The lack of polymorphonuclear neutrophils, lymphocytes, and histiocytes at the...titer of 1:1,000 on a partially purified CIF A containing bone extract. The **antibodies** also reacted with **TGF-beta** from platelets, as expected, since the N-terminal sequence is identical.

The competitive ELISA method...

1/KWIC/137 (Item 136 from file: 654)  
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...Implant-associated inflammation was negligible compared with the control implants. A dense collagenous connective tissue **matrix** was evident throughout the implant. Morphologically, the fibroblasts appeared to be metabolically less active than at earlier time points.

These histological observations demonstrate that CIF **inhibits** inflammatory cell function in vivo. The lack of polymorphonuclear neutrophils, lymphocytes, and histiocytes at the...titer of 1:1,000 on a partially purified CIF-A containing bone extract. The **antibodies** also reacted with **TGF-beta** from platelets, as expected, since the N-terminal sequence is identical.

The competitive ELISA method...

t sl5/3/all

15/3/1 (Item 1 from file: 5)  
DIALOG(R)File 5:BIOSIS Previews(R)  
(c) 1999 BIOSIS. All rts. reserv.

11133000 BIOSIS NO.: 199799754145  
Inflammatory and anti-inflammatory cytokines in **glomerulonephritis**.

AUTHOR: Lakkis F G(a); Dai Z  
AUTHOR ADDRESS: (a)Renal Div., Emory Univ. Sch. Med., Atlanta, GA, USA

JOURNAL: EOS-Rivista di Immunologia ed Immunofarmacologia 17 (1):p7-11  
1997  
ISSN: 0392-6699  
DOCUMENT TYPE: Literature Review  
RECORD TYPE: Abstract  
LANGUAGE: English  
SUMMARY LANGUAGE: English; Italian

15/3/2 (Item 1 from file: 73)  
DIALOG(R)File 73:EMBASE  
(c) 1999 Elsevier Science B.V. All rts. reserv.

05490799 EMBASE No: 1993258898  
Expression of cytokines and growth factors in human glomerulonephritides  
Waldherr R.; Noronha I.L.; Niemir Z.; Kruger C.; Stein H.; Stumm G.  
Department of Pathology, University of Heidelberg, Im Neuenheimer Feld  
220, D-69120 Heidelberg Germany  
Pediatric Nephrology ( PEDIATR. NEPHROL. ) (Germany) 1993, 7/4 (471-478)  
  
CODEN: PEDNE ISSN: 0931-041X  
DOCUMENT TYPE: Journal; Review  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

15/3/3 (Item 2 from file: 73)  
DIALOG(R)File 73:EMBASE  
(c) 1999 Elsevier Science B.V. All rts. reserv.

05398519 EMBASE No: 1993166618  
Factors involved in the regulation of mesangial cell proliferation in  
vitro and in vivo  
Floege J.; Eng E.; Young B.A.; Johnson R.J.  
Division of Nephrology, University of Washington, Seattle, WA 98195  
United States  
Kidney International, Supplement ( KIDNEY INT. SUPPL. ) (United States)  
1993, -/39 (S-47-S-54)

CODEN: KISUD ISSN: 0098-6577  
DOCUMENT TYPE: Journal; Conference Paper  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

15/3/4 (Item 1 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
(c) format only 1999 Dialog Corporation. All rts. reserv.

08135032 95201220

Experimental insights into the tubulointerstitial disease accompanying primary glomerular lesions [editorial]

Eddy AA

J Am Soc Nephrol (UNITED STATES) Dec 1994, 5 (6) p1273-87, ISSN 1046-6673 Journal Code: A6H

Languages: ENGLISH

Document type: EDITORIAL; REVIEW; REVIEW, TUTORIAL

15/3/5 (Item 1 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

03024224

Utility  
APOPTOSIS INDUCED BY SHIGELLA IPAB

PATENT NO.: 5,972,899  
ISSUED: October 26, 1999 (19991026)  
INVENTOR(s): Zychlinsky, Arturo, New York, NY (New York), US (United States of America)  
Chen, Yajing, Elmhurst, NY (New York), US (United States of America)  
ASSIGNEE(s): New York University, (A U.S. Company or Corporation ), New York, NY (New York), US (United States of America)  
APPL. NO.: 8-591,079  
FILED: January 25, 1996 (19960125)  
FULL TEXT: 3939 lines

15/3/6 (Item 2 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

03019877

Utility  
CELL-CYCLE REGULATORY PROTEINS, AND USES RELATED THERETO

PATENT NO.: 5,968,821  
ISSUED: October 19, 1999 (19991019)  
INVENTOR(s): Beach, David H., Huntington Bay, NY (New York), US (United States of America)  
Demetrick, Douglas J., E. Northport, NY (New York), US (United States of America)  
Serrano, Manuel, Mill Neck, NY (New York), US (United States of America)  
Hannon, Gregory J., Huntington, NY (New York), US (United States of America)  
ASSIGNEE(s): Cold Spring Harbor Laboratories, Inc , (A U.S. Company or Corporation), Cold Spring Harbor, NY (New York), US (United States of America)  
APPL. NO.: 8-893,274  
FILED: July 15, 1997 (19970715)

#### RELATED APPLICATIONS

This application is a continuation application of Ser. No. 08-306,511 filed on Sep. 14, 1994, which is a continuation-in-part of U.S. Ser. No. 08-248,812 filed May 25, 1994 and entitled "Cell-cycle Regulatory Protein, and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 08-227,371 filed Apr. 14, 1994 and entitled "Cell-cycle Regulatory Protein, and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 08-154,915 filed Nov. 18, 1993 and entitled "Cyclin Complex Rearrangements and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 07-991,997 filed Dec. 17, 1992 and entitled "Cyclin Complex Rearrangements and Uses Related Thereto", abandoned, which is a continuation-in-part of

U.S. Ser. No. 07-963,308 filed Oct. 16, 1992 and entitled "D-Type Cyclin and Uses Related Thereto". The teachings of U.S. Ser. Nos. 08-248,812, 08-227,371, 08-154,915, 07-991,997, 07-963,308 and related PCT publication US93-09945 are incorporated herein by reference.

#### FUNDING

Work described herein was supported by National Institutes NIH Grant Nos. ROI GM39620, ROI CA63518 and ROI CA68040 of Health Grant and the Howard Hughes Medical Institute. The United States Government has certain rights in the invention.

FULL TEXT: 3685 lines

15/3/7 (Item 3 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

03019817

Utility  
UBIQUITIN CONJUGATING ENZYMES

PATENT NO.: 5,968,761  
ISSUED: October 19, 1999 (19991019)  
INVENTOR(s): Rolfe, Mark, Newton Upper Falls, MA (Massachusetts), US  
(United States of America)  
Chiu, Maria Isabel, Boston, MA (Massachusetts), US (United States of America)  
Cottarel, Guillaume, West Roxbury, MA (Massachusetts), US (United States of America)  
Berlin, Vivian, Dunstable, MA (Massachusetts), US (United States of America)  
Damagnez, Veronique, Cambridge, MA (Massachusetts), US (United States of America)  
Draetta, Giulio, Winchester, MA (Massachusetts), US (United States of America)  
ASSIGNEE(s): Mitotix, Inc , (A U.S. Company or Corporation), Cambridge, MA (Massachusetts), US (United States of America)  
[Assignee Code(s): 36847]  
APPL. NO.: 8-486,663  
FILED: June 07, 1995 (19950607)

#### RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Ser. No. 08-250,795, filed May 27, 1994 entitled "Immunosuppressant Target Proteins", and is a continuation-in-part of U.S. Ser. No. 08-305,520, filed Sep. 13, 1994, now U.S. Pat No. 5,744,343, entitled "Ubiquitin Conjugating Enzymes", which is a continuation-in-part of U.S. Ser. No. 08-247,904, filed May 23, 1994 entitled "Human Ubiquitin Conjugating Enzyme", which is a continuation-in-part of U.S. Ser. No. 08-176,937, filed Jan. 4, 1994 now abandoned, entitled "Assay and Reagents for Detecting Inhibitors of Ubiquitin-dependent Degredation of Cell Cycle Regulatory Proteins", the specification of which are incorporated by reference herein.

FULL TEXT: 5119 lines

15/3/8 (Item 4 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

03012658

Utility

CELL-CYCLE REGULATORY PROTEINS, AND USES RELATED THERETO

PATENT NO.: 5,962,316  
ISSUED: October 05, 1999 (19991005)  
INVENTOR(s): Beach, David H., Huntington Bay, NY (New York), US (United States of America)  
Demetrick, Douglas J., Northport, NY (New York), US (United States of America)  
Serrano, Manuel, Mill Neck, NY (New York), US (United States of America)  
Hannon, Gregory J., Huntington, NY (New York), US (United States of America)  
ASSIGNEE(s): Cold Spring Harbor Laboratory, (A U.S. Company or Corporation), Cold Spring Harbor, NY (New York), US (United States of America)  
[Assignee Code(s): 797]  
APPL. NO.: 8-306,511  
FILED: September 14, 1994 (19940914)  
RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Ser. No. 08-248,812 filed May 25, 1994 and entitled "Cell-cycle Regulatory Protein, and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 08-227,371 filed Apr. 14, 1994 and entitled "Cell-cycle Regulatory Protein, and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 08-154,915 filed Nov. 18, 1993 and entitled "Cyclin Complex Rearrangements and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 07-991,997 filed Dec. 17, 1992 and entitled "Cyclin Complex Rearrangements and Uses Related Thereto", now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-963,308 filed Oct. 16, 1992 and entitled "D-Type Cyclin and Uses Related Thereto". The teachings of U.S. Ser. Nos. 08-248,812, 08-227,371, 08-154,915, 07-991,997, 07-963,308 and related PCT publication US 93-09945 are incorporated herein by reference.

FUNDING

Work described herein was supported by National Institutes under NIH Grant Nos. R01 GM39620 and R01 CA63518 of Health Grant and the Howard Hughes Medical Institute. The United States Government has certain rights in the invention.

FULL TEXT: 3628 lines

15/3/9 (Item 5 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

03008923

Utility  
OCULAR THERAPY IN KERATOCONJUNCTIVITIS SICCA USING TOPICALLY APPLIED ANDROGENS OF TGF- $\beta$ 1.

PATENT NO.: 5,958,912  
ISSUED: September 28, 1999 (19990928)  
INVENTOR(s): Sullivan, David A., Acton, MA (Massachusetts), US (United States of America)  
ASSIGNEE(s): The Schepens Eye Research Institute, Inc , (A U.S. Company or Corporation), Boston, MA (Massachusetts), US (United States of America)  
[Assignee Code(s): 39547]  
APPL. NO.: 8-971,768  
FILED: November 17, 1997 (19971117)

This application is a continuation-in-part of Sullivan, U.S. patent

application Ser. No. 08-477,301, filed Jun. 7, 1995, now U.S. Pat. No. 5,688,765 which was a continuation-in-part of Sullivan, U.S. patent application Ser. No. 08-124,842, filed Sep. 21, 1993, now U.S. Pat. No. 5,620,921, which was a continuation under 37 CFR 1.62 of U.S. patent application Ser. No. 07-871,657, filed Apr. 21, 1992, now abandoned the whole of which are hereby incorporated by reference herein.

#### GOVERNMENT RIGHTS

Part of the work leading to this invention was made with United States Government funds under Grant No. EY05612 from the National Institutes of Health. Therefore, the U.S. Government has certain rights in this invention.

FULL TEXT: 1278 lines

15/3/10 (Item 6 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

03008702

Utility  
HUMAN TSC--22 HOMOLOG

PATENT NO.: 5,958,690  
ISSUED: September 28, 1999 (19990928)  
INVENTOR(s): Hillman, Jennifer L., Mountain View, CA (California), US  
(United States of America)  
Lal, Preeti, Santa Clara, CA (California), US (United States of America)  
Shah, Purvi, Sunnyvale, CA (California), US (United States of America)  
ASSIGNEE(s): Incyte Pharmaceuticals, Inc , (A U.S. Company or Corporation),  
Palo Alto, CA (California), US (United States of America)  
[Assignee Code(s): 27511]  
APPL. NO.: 8-889,337  
FILED: July 08, 1997 (19970708)  
FULL TEXT: 1986 lines

15/3/11 (Item 7 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

03001627

Utility  
DNA ENCODING THE CHEMOTACTIC CYTOKINE III

PATENT NO.: 5,952,197  
ISSUED: September 14, 1999 (19990914)  
INVENTOR(s): Ni, Jian, Rockville, MD (Maryland), US (United States of America)  
Gentz, Reiner, Silver Spring, MD (Maryland), US (United States of America)  
Yu, Guo-Liang, Darnestown, MD (Maryland), US (United States of America)  
Su, Jeffrey, Gaithersburg, MD (Maryland), US (United States of America)  
Dillon, Patrick J., Gaithersburg, MD (Maryland), US (United States of America)  
ASSIGNEE(s): Human Genome Sciences, Inc , (A U.S. Company or Corporation),  
Rockville, MD (Maryland), US (United States of America)  
[Assignee Code(s): 38350]  
APPL. NO.: 8-812,003

FILED: March 05, 1997 (19970305)

This application claims benefit of 35 U.S.C. section 120 based on copending U.S. Provisional Application Ser. No. 60-013,609, filed on Mar. 5, 1996.

FULL TEXT: 2170 lines

15/3/12 (Item 8 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02987666

Utility  
MESANGIAL CELL LINES AS MODELS FOR THE STUDY AND TREATMENT OF DIABETIC TISSUE COMPLICATIONS

PATENT NO.: 5,939,275  
ISSUED: August 17, 1999 (19990817)  
INVENTOR(s): Heilig, Charles W., 28 Whittler's Ridge, Pittsford, NY (New York), US (United States of America), 14534  
Freytag, Svend O., 1778 Bournmouth, Grosse Point Woods, MI (Michigan), US (United States of America), 48202  
Riser, Bruce L., 409 Brewer St., Marshall, MI (Michigan), US (United States of America), 49068  
[Assignee Code(s): 68000]  
APPL. NO.: 8-522,571  
FILED: September 01, 1995 (19950901)

#### GRANT REFERENCE

Research in this application was supported in part by grants from the National Institutes of Health, K08 DK01953 (Heilig), CA64295 (Freytag) and a grant from the Juvenile Diabetes Foundation International, 1921461 (Riser). The Government and the Juvenile Diabetes Foundation International have certain rights in the invention.

FULL TEXT: 2162 lines

15/3/13 (Item 9 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02976685

Utility  
HUMAN ACTVA-ORF4-LIKE PROTEIN

PATENT NO.: 5,928,894  
ISSUED: July 27, 1999 (19990727)  
INVENTOR(s): Lal, Preeti, Santa Clara, CA (California), US (United States of America)  
Tang, Tom, San Jose, CA (California), US (United States of America)  
Corley, Neil C., Mountain View, CA (California), US (United States of America)  
Shah, Purvi, Sunnyvale, CA (California), US (United States of America)  
ASSIGNEE(s): Incyte Pharmaceuticals, Inc , (A U.S. Company or Corporation), Palo Alto, CA (California), US (United States of America)  
[Assignee Code(s): 27511]  
APPL. NO.: 8-923,856  
FILED: September 03, 1997 (19970903)  
FULL TEXT: 2209 lines



15/3/14 (Item 10 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02967071

Utility  
METHOD FOR INHIBITING EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE WITH  
TETRACYCLINE

PATENT NO.: 5,919,775  
ISSUED: July 06, 1999 (19990706)  
INVENTOR(s): Amin, Ashok R., Union, NJ (New Jersey), US (United States of America)  
Abramson, Steven B., Rye, NY (New York), US (United States of America)  
Golub, Lorne M., Smithtown, NY (New York), US (United States of America)  
Ramamurthy, Nungavaram S., Smithtown, NY (New York), US (United States of America)  
McNamara, Thomas F., Port Jefferson, NY (New York), US (United States of America)  
Greenwald, Robert A., Melville, NY (New York), US (United States of America)  
Trachtman, Howard, New Rochelle, NY (New York), US (United States of America)  
ASSIGNEE(s): Hospital For Joint Diseases, (A U.S. Company or Corporation), New York, NY (New York), US (United States of America)  
The Research Foundation of The State University of New York, (A U.S. Company or Corporation), Stony Brook, NY (New York), US (United States of America)  
[Assignee Code(s): 5711; 45654]  
APPL. NO.: 9-61,286  
FILED: April 16, 1998 (19980416)

This application is a division of Ser. No. 08-697,815, filed Aug. 30, 1996, now U.S. Pat. No. 5,789,395.

FULL TEXT: 1276 lines

15/3/15 (Item 11 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02964146

Utility  
METHODS OF TREATING INFLAMMATION AND COMPOSITIONS THEREFOR

PATENT NO.: 5,917,014  
ISSUED: June 29, 1999 (19990629)  
INVENTOR(s): McFadden, D. Grant, Edmonton, CA (Canada)  
Lucas, Alexandra, Edmonton, CA (Canada)  
ASSIGNEE(s): Viron Therapeutics, Inc , (A Non-U.S. Company or Corporation), London, CA (Canada)  
APPL. NO.: 8-468,865  
FILED: June 06, 1995 (19950606)

This is a continuation of copending application Ser. No. 08-411,043, filed on Mar. 27, 1995.

FULL TEXT: 1932 lines

15/3/16 (Item 12 from file: 654)  
DIALOG(R)File 654:US Pat.Full.

02925465

Utility

COMPOSITIONS AND METHODS FOR TREATING AND PREVENTING PATHOLOGIES INCLUDING CANCER

PATENT NO.: 5,883,124

ISSUED: March 16, 1999 (19990316)

INVENTOR(s): Samid, Dvorit, Rockville, MD (Maryland), US (United States of America)

ASSIGNEE(s): National Starch & Chemical Investment Holding Coporation, (A U.S. Company or Corporation ), Wilmington, DE (Delaware), US (United States of America)

APPL. NO.: 8-484,615

FILED: June 07, 1995 (19950607)

PRIORITY: 9414853, GB (United Kingdom), July 22, 1994 (19940722)

9511880, GB (United Kingdom), June 10, 1995 (19950610)

This application is a divisional of U.S. application Ser. No. 08-207,521, filed Mar. 7, 1994, pending, which is (1) a continuation-in-part of Applicant's Ser. No. 08-135,661, filed Oct. 12, 1993, pending and also is (2) a continuation-in-part of Applicant's U.S. Ser. No. 07-779,744, filed Oct. 21, 1991, and now abandoned, the contents of all of which are hereby incorporated by this reference.

FULL TEXT: 7322 lines

15/3/17 (Item 13 from file: 654)

DIALOG(R)File 654:US Pat.Full.

(c) format only 1999 The Dialog Corp. All rts. reserv.

02910352

Utility

METHOD OF DETERMINING DNA SEQUENCE PREFERENCE OF A DNA-BINDING MOLECULE

PATENT NO.: 5,869,241

ISSUED: February 09, 1999 (19990209)

INVENTOR(s): Edwards, Cynthia A., Menlo Park, CA (California), US (United States of America)

Cantor, Charles R., Boston, MA (Massachusettes), US (United States of America)

Andrews, Beth M., Maynard, MA (Massachusettes), US (United States of America)

Turin, Lisa M., Redwood City, CA (California), US (United States of America)

Fry, Kirk E., Palo Alto, CA (California), US (United States of America)

ASSIGNEE(s): Genelabs Technologies, Inc , (A U.S. Company or Corporation), Redwood City, CA (California), US (United States of America)  
[Assignee Code(s): 33390]

APPL. NO.: 8-475,228

FILED: June 07, 1995 (19950607)

This application is a divisional of application Ser. No. 08-171,389 filed 20 Dec. 1993 and now U.S. Pat. No. 5,578,444, herein incorporated by reference, which is a continuation-in-part of application Ser. No. 08-123,936 filed 17 Sep. 1993 and now U.S. Pat. No. 5,726,014, herein incorporated by reference, which is a continuation-in-part of application Ser. No. 07-996,783 filed 23 Dec. 1992 and now U.S. Pat. No. 5,693,463, herein incorporated by reference, which is a continuation-in-part of application Ser. No. 07-723,618 filed 27 Jun. 1991, now abandoned, and being prosecuted as co-pending, co-owned file-wrapper continuation 08-081,070, filed 22 Jun. 1993, now U.S. Pat. No. 5,306,619, herein incorporated by reference.

FULL TEXT: 14067 lines

15/3/18 (Item 14 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02890856

Utility  
PHENYLACETATE AND DERIVATIVES ALONE OR IN COMBINATION WITH OTHER COMPOUNDS  
AGAINST NEOPLASTIC CONDITIONS AND OTHER DISORDERS

PATENT NO.: 5,852,056  
ISSUED: December 22, 1998 (19981222)  
INVENTOR(s): Samid, Dvorit, Rockville, MD (Maryland), US (United States of America)  
ASSIGNEE(s): The United States of America as represented by the Department of Health and Human Services, (A U.S. Government Agency), Washington, DC (District of Columbia, US (United States of America)  
[Assignee Code(s): 6814]  
APPL. NO.: 8-633,833  
FILED: April 10, 1996 (19960410)  
PCT: PCT-US94-11492 (WO 94US11492)  
Section 371 Date: April 10, 1996 (19960410)  
Section 102(e) Date: April 10, 1996 (19960410)  
Filing Date: October 12, 1994 (19941012)  
Publication Number: WO95-10271 (WO 9510271)  
Publication Date: April 20, 1995 (19950420)

This application is a national stage filing under 35 U.S.C. selection 371 of International Application PCT-US94-11492, filed Oct. 12, 1994, which is a continuation of U.S. Ser. No. 08-207,521, filed Mar. 7, 1994, and now U.S. Pat. No. 5,605,930, and, U.S. Ser. No. 08-135,661, filed Oct. 12, 1993, and now U.S. Pat. No. 5,635,532, both of which in turn are continuations-in-part of U.S. Ser. No. 07-779,744, filed Oct. 21, 1991, and now abandoned.

FULL TEXT: 4735 lines

15/3/19 (Item 15 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02890618

Utility  
MCH4 AND MCH5, APOPTOTIC PROTEASES

PATENT NO.: 5,851,815  
ISSUED: December 22, 1998 (19981222)  
INVENTOR(s): Alnemri, Emad S., Ambler, PA (Pennsylvania), US (United States of America)  
Fernandes-Alnemri, Teresa, Ambler, PA (Pennsylvania), US (United States of America)  
Litwack, Gerald, Wynnewood, PA (Pennsylvania), US (United States of America)  
Armstrong, Robert, San Diego, CA (California), US (United States of America)  
Tomaselli, Kevin, La Jolla, CA (California), US (United States of America)  
ASSIGNEE(s): IDUN Pharmaceuticals, Inc, (A U.S. Company or Corporation), La Jolla, CA (California), US (United States of America)  
[Assignee Code(s): 46396]  
APPL. NO.: 8-618,408

FILED: March 19, 1996 (19960319)

This invention was made with government support under grants AI 35035-01 from the National Institutes of Health. Accordingly, the government has certain rights to this invention.

FULL TEXT: 2238 lines

15/3/20 (Item 16 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02888470

Utility  
TRANSGENIC MOUSE DEFICIENT IN INDUCIBLE NITRIC OXIDE SYNTHASE

PATENT NO.: 5,850,004  
ISSUED: December 15, 1998 (19981215)  
INVENTOR(s): MacMicking, John, New York, NY (New York), US (United States of America)  
Nathan, Carl, Larchmont, NY (New York), US (United States of America)  
Mudgett, John S., Scotch Plains, NJ (New Jersey), US (United States of America)  
ASSIGNEE(s): Cornell Research Foundation, Inc , (A U.S. Company or Corporation), Ithaca, NY (New York), US (United States of America)  
Merck & Co Inc , (A U.S. Company or Corporation), Rahway, NJ (New Jersey), US (United States of America)  
[Assignee Code(s): 20656; 54136]  
APPL. NO.: 8-808,191  
FILED: February 28, 1997 (19970228)

This application is a continuation of application Ser. No. 08-284,898 filed Aug. 2, 1994 abandoned.

This invention was made with partial Government support under Grant Nos. HL51967 and AI34543 from the National Institutes of Health. The Government has certain rights in this invention.

FULL TEXT: 1283 lines

15/3/21 (Item 17 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02881864

Utility  
COMPOSITIONS AND METHODS FOR TREATING AND PREVENTING PATHOLOGIES INCLUDING CANCER

PATENT NO.: 5,843,994  
ISSUED: December 01, 1998 (19981201)  
INVENTOR(s): Samid, Dvorit, Rockville, MD (Maryland), US (United States of America)  
ASSIGNEE(s): The United States of America as represeneted by the Department of Health and Human Services, (A U.S. Government Agency), Washington, DC (District of Columbia, US (United States of America)  
APPL. NO.: 8-478,264  
FILED: June 07, 1995 (19950607)

This application is a divisional of U.S. application Ser. No. 08-207,521, filed Mar. 7, 1994, now U.S. Pat. No. 5,605,930 which, in turn, is (1) a

continuation-in-part of Applicant's U.S. Ser. No. 08-135,661, filed Oct. 12, 1993, abandoned, which is (2) a continuation-in-part of Applicant's U.S. Ser. No. 07-779,744, filed Oct. 21, 1991, abandoned, the contents of all parent applications are hereby incorporated by this reference.

FULL TEXT: 7541 lines

15/3/22 (Item 18 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02875103

Utility  
NUCLEIC ACIDS MOLECULES ENCODING CASPASE-8H AND CASPASE-8I

PATENT NO.: 5,837,837  
ISSUED: November 17, 1998 (19981117)  
INVENTOR(s): Hunter, John J., Cambridge, MA (Massachusetts), US (United States of America)  
Shyjan, Andrew W., Nahant, MA (Massachusetts), US (United States of America)  
Wong, Grace H. W., Brookline, MA (Massachusetts), US (United States of America)  
ASSIGNEE(s): Millennium Pharmaceuticals, Inc , (A U.S. Company or Corporation), Cambridge, MA (Massachusetts), US (United States of America)  
[Assignee Code(s): 41994]  
APPL. NO.: 8-807,200  
FILED: February 27, 1997 (19970227)  
FULL TEXT: 1989 lines

15/3/23 (Item 19 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02874570

Utility  
USE OF INTERLEUKIN-10 ANALOGS FOR ANTAGONISTS TO TREAT ENDOTOXIN- OR SUPERANTIGEN-INDUCED TOXICITY

PATENT NO.: 5,837,293  
ISSUED: November 17, 1998 (19981117)  
INVENTOR(s): De Waal Malefyt, Rene, Sunnyvale, CA (California), US (United States of America)  
Howard, Maureen, Los Altos Hills, CA (California), US (United States of America)  
Hsu, Di-Hwei, Sunnyvale, CA (California), US (United States of America)  
Ishida, Hiroshi, Kyoto, JP (Japan)  
O'Garra, Anne, Palo Alto, CA (California), US (United States of America)  
Spits, Hergen, Badhoevedorp, NL (Netherlands)  
Zlotnik, Albert, Palo Alto, CA (California), US (United States of America)  
ASSIGNEE(s): Schering Corporation, (A U.S. Company or Corporation), Kenilworth, NJ (New Jersey), US (United States of America)  
[Assignee Code(s): 74480]  
APPL. NO.: 8-481,560  
FILED: June 07, 1995 (19950607)

This is a divisional of U.S. Ser. No. 08-410,654, filed Mar. 24, 1995, which is a continuation-in-part of U.S. Ser. No. 08-229,854 filed Apr. 19, 1994, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-926,853 filed Aug. 6, 1992, now abandoned, which is a

continuation-in-part of U.S. Ser. No. 07-742,129 filed Aug. 6, 1991, now abandoned.

FULL TEXT: 5214 lines

15/3/24 (Item 20 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02874513

Utility  
USE OF AN INTERLEUKIN-10 ANTAGONIST TO TREAT A B CELL MEDIATED AUTOIMMUNE DISORDER

PATENT NO.: 5,837,232  
ISSUED: November 17, 1998 (19981117)  
INVENTOR(s): De Waal Malefyt, Rene, Sunnyvale, CA (California), US (United States of America)  
Howard, Maureen, Los Altos Hills, CA (California), US (United States of America)  
Hsu, Di-Hwei, Sunnyvale, CA (California), US (United States of America)  
Ishida, Hiroshi, Kyoto, JP (Japan)  
O'Garra, Anne, Palo Alto, CA (California), US (United States of America)  
Spits, Hergen, Badhoevedorp, NL (Netherlands)  
Zlotnik, Albert, Palo Alto, CA (California), US (United States of America)  
ASSIGNEE(s): Schering Corporation, (A U.S. Company or Corporation), Kenilworth, NJ (New Jersey), US (United States of America)  
[Assignee Code(s): 74480]  
APPL. NO.: 8-474,851  
FILED: June 07, 1995 (19950607)

This is a divisional of U.S. Ser. No. 08-410,654, filed Mar. 24, 1995, which is a continuation of U.S. Ser. No. 08-229,854 filed Apr. 19, 1994, now abandoned, which is a continuation of U.S. Ser. No. 07-926,853 filed Aug. 6, 1992, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-742,129 filed Aug. 6, 1991, now abandoned.

FULL TEXT: 4337 lines

15/3/25 (Item 21 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02871201

Utility  
DNA ENCODING A TRANSFORMING GROWTH FACTOR-.BETA. RECEPTOR ASSOCIATED PROTEIN

PATENT NO.: 5,834,240  
ISSUED: November 10, 1998 (19981110)  
INVENTOR(s): Bandman, Olga, Mountain View, CA (California), US (United States of America)  
Lal, Preeti, Sunnyvale, CA (California), US (United States of America)  
ASSIGNEE(s): Incyte Pharmaceuticals, Inc , (A U.S. Company or Corporation), Palo Alto, CA (California), US (United States of America)  
[Assignee Code(s): 27511]  
APPL. NO.: 8-828,922  
FILED: March 28, 1997 (19970328)  
FULL TEXT: 1838 lines

15/3/26 (Item 22 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02870946

Utility

USE OF INTERLEUKIN-10 (IL-10) TO TREAT ENDOTOXIN- OR SUPERANTIGEN-INDUCED TOXICITY

PATENT NO.: 5,833,976  
ISSUED: November 10, 1998 (19981110)  
INVENTOR(s): Malefyt, Rene de Waal, Mountain View, CA (California), US (United States of America)  
Howard, Maureen, Los Altos Hills, CA (California), US (United States of America)  
Hsu, Di-Hwei, Palo Alto, CA (California), US (United States of America)  
Ishida, Hiroshi, Wakayama, JP (Japan)  
O'Garra, Anne, Palo Alto, CA (California), US (United States of America)  
Spits, Hergen, Los Altos, CA (California), US (United States of America)  
Zlotnik, Albert, Palo Alto, CA (California), US (United States of America)  
ASSIGNEE(s): Schering Corporation, (A U.S. Company or Corporation), Kenilworth, NJ (New Jersey), US (United States of America)  
[Assignee Code(s): 74480]  
APPL. NO.: 8-410,654  
FILED: March 24, 1995 (19950324)

This is a continuation of U.S. Ser. No. 08-229,854 filed Apr. 19, 1994, now abandoned, which is a continuation of U.S. Ser. No. 07-926,853 filed Aug. 6, 1992, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-742,129 filed Aug. 6, 1991, now abandoned.

FULL TEXT: 4235 lines

15/3/27 (Item 23 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02867514

Utility

SOLUBLE TGF- $\beta$ -BINDING ENDOGLIN POLYPEPTIDES AND HOMODIMERS

PATENT NO.: 5,830,847  
ISSUED: November 03, 1998 (19981103)  
INVENTOR(s): Letarte, Michelle, Toronto, CA (Canada)  
Massague, Joan, New York, NY (New York), US (United States of America)  
Bernabeu, Carmelo, Madrid, ES (Spain)  
Cheifetz, Sela, Concord, CA (Canada)  
ASSIGNEE(s): HSC Research & Development Limited Partnership, (A Non-U.S. Company or Corporation), CA (Canada)  
[Assignee Code(s): 36023]  
APPL. NO.: 7-968,953  
FILED: October 30, 1992 (19921030)

This invention was made in part with Government support under Grant No. CA 34610, from the National Institutes of Health. The Government may have certain rights in this invention.

FULL TEXT: 852 lines

15/3/28 (Item 24 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02867344

Utility  
METHOD FOR ASSAYING FOR MODULATORS OF CYTOKINES OF THE TFG .BETA.  
SUPERFAMILY

PATENT NO.: 5,830,671  
ISSUED: November 03, 1998 (19981103)  
INVENTOR(s): Dennis, James W., Etobicoke, CA (Canada)  
Demetriou, Michael, Toronto, CA (Canada)  
ASSIGNEE(s): Mount Sinai Hospital Corporation, (A Non-U.S. Company or  
Corporation), Toronto, CA (Canada)  
[Assignee Code(s): 21289]  
APPL. NO.: 8-854,768  
FILED: May 12, 1997 (19970512)

This is a Continuation of application Ser. No. 08-237,715, filed May 4, 1994.

FULL TEXT: 1449 lines

15/3/29 (Item 25 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02860761

Utility  
ANTI-TRANSFORMING GROWTH FACTOR-.BETA. GENE THERAPY

PATENT NO.: 5,824,655  
ISSUED: October 20, 1998 (19981020)  
INVENTOR(s): Border, Wayne A., Salt Lake City, UT (Utah), US (United States  
of America)  
ASSIGNEE(s): The University of Utah, (A U.S. Company or Corporation), Salt  
Lake City, UT (Utah), US (United States of America)  
[Assignee Code(s): 88042]  
APPL. NO.: 8-389,887  
FILED: February 15, 1995 (19950215)

The present invention was supported in part by Grant DK43609 from the National Institute of Diabetes and Digestinal and Kidney Diseases. The U.S. government may have certain rights in this invention.

FULL TEXT: 1414 lines

15/3/30 (Item 26 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02857071

Utility  
MODULATORS OF CYTOKINES OF THE TGF .BETA. SUPERFAMILY

PATENT NO.: 5,821,227  
ISSUED: October 13, 1998 (19981013)  
INVENTOR(s): Dennis, James W., Etobicoke, CA (Canada)  
Demetriou, Michael, Toronto, CA (Canada)  
ASSIGNEE(s): Mount Sinai Hospital Corporation, (A Non-U.S. Company or  
Corporation), Toronto, CA (Canada)  
[Assignee Code(s): 21289]  
APPL. NO.: 8-483,926



CROSS REFERENCE TO RELATED APPLICATION

This is a continuation of U.S. patent application Ser. No. 08-237,715, filed May 4, 1994, now abandoned.

FULL TEXT: 1702 lines

15/3/31 (Item 27 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02842926

Utility  
PEPTIDES WHICH ARE CLEAVED BY C-PROTEINASE

PATENT NO.: 5,807,981  
ISSUED: September 15, 1998 (19980915)  
INVENTOR(s): Brenner, Mitch, Mountain View, CA (California), US (United States of America)  
ASSIGNEE(s): FibroGen Inc , (A U.S. Company or Corporation), South San Francisco, CA (California), US (United States of America)  
APPL. NO.: 8-572,225  
FILED: December 13, 1995 (19951213)  
FULL TEXT: 1433 lines

15/3/32 (Item 28 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02822888

Utility  
METHOD OF USING TETRACYCLINE COMPOUNDS FOR INHIBITION OF ENDOGENOUS NITRIC OXIDE PRODUCTION  
[Enzyme inhibitors, treatment of osteoarthritis, nonbactericidal]

PATENT NO.: 5,789,395  
ISSUED: August 04, 1998 (19980804)  
INVENTOR(s): Amin, Ashok R., Union, NJ (New Jersey), US (United States of America)  
Abramson, Steven B., Rye, NY (New York), US (United States of America)  
Golub, Lorne M., Smithtown, NY (New York), US (United States of America)  
Ramamurthy, Nungavaram S., Smithtown, NY (New York), US (United States of America)  
McNamara, Thomas F., Port Jefferson, NY (New York), US (United States of America)  
Greenwald, Robert A., Melville, NY (New York), US (United States of America)  
Trachtman, Howard, New Rochelle, NY (New York), US (United States of America)  
ASSIGNEE(s): Hospital for Joint Diseases, (A U.S. Company or Corporation), New York, NY (New York), US (United States of America)  
The Research Foundation of State University of New York, (A U.S. Company or Corporation), Albany, NY (New York), US (United States of America)  
[Assignee Code(s): 5711; 45654]  
APPL. NO.: 8-697,815  
FILED: August 30, 1996 (19960830)  
FULL TEXT: 1281 lines

15/3/33 (Item 29 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02819535

Utility

MCH4 AND MCH5, APOPTOTIC PROTEASE, NUCLEIC ACIDS ENCODING AND METHODS OF USE

PATENT NO.: 5,786,173  
ISSUED: July 28, 1998 (19980728)  
INVENTOR(s): Alnemri, Emad S., Ambler, PA (Pennsylvania), US (United States of America)  
Fernandes-Alnemri, Teresa, Ambler, PA (Pennsylvania), US (United States of America)  
Litwack, Gerald, Wynnewood, PA (Pennsylvania), US (United States of America)  
Armstrong, Robert, San Diego, CA (California), US (United States of America)  
Tomaselli, Kevin, La Jolla, CA (California), US (United States of America)  
ASSIGNEE(s): Idun Pharmaceuticals, Inc , (A U.S. Company or Corporation),  
La Jolla, CA (California), US (United States of America)  
APPL. NO.: 8-665,220  
FILED: June 14, 1996 (19960614)

This application is a continuation-in-part of U.S. Ser. No. 08-618,408, filed on Mar. 19, 1996.

This invention was made with government support under grants AI 35035-01 from the National Institutes of Health. Accordingly, the government has certain rights to this invention.

FULL TEXT: 2591 lines

15/3/34 (Item 30 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02792326

Utility

OSTEOARTHRITIS-ASSOCIATED INDUCABLE ISOFORM OF NITRIC OXIDE SYNTHETASE

PATENT NO.: 5,759,836  
ISSUED: June 02, 1998 (19980602)  
INVENTOR(s): Amin, Ashok R., Union, NJ (New Jersey), US (United States of America)  
Abramson, Steven B., Rye, NY (New York), US (United States of America)  
ASSIGNEE(s): Hospital For Joint Diseases, (A U.S. Company or Corporation),  
New York, NY (New York), US (United States of America)  
APPL. NO.: 8-410,739  
FILED: March 27, 1995 (19950327)  
FULL TEXT: 1966 lines

15/3/35 (Item 31 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02775747

Utility

SEQUENCE-DIRECTED DNA-BINDING MOLECULES COMPOSITIONS AND METHODS

PATENT NO.: 5,744,131  
ISSUED: April 28, 1998 (19980428)  
INVENTOR(s): Edwards, Cynthia A., Menlo Park, CA (California), US (United States of America)  
Fry, Kirk E., Palo Alto, CA (California), US (United States of America)  
Cantor, Charles R., Boston, MA (Massachusetts), US (United States of America)  
Andrews, Beth M., Maynard, MA (Massachusetts), US (United States of America)  
ASSIGNEE(s): Genelabs Technologies, Inc , (A U.S. Company or Corporation), Redwood City, CA (California), US (United States of America)  
[Assignee Code(s): 33390]  
APPL. NO.: 8-476,876  
FILED: June 07, 1995 (19950607)

This application is a division of application Ser. No. 07-996,783 filed Dec. 23, 1992, herein incorporated by reference, which is a continuation-in-part of co-owned, U.S. application Ser. No. 07-723,618, filed 27 Jun. 1991 now abandoned.

FULL TEXT: 5877 lines

15/3/36 (Item 32 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02770253

Utility  
SEQUENCE-DIRECTED DNA-BINDING MOLECULES COMPOSITIONS AND METHODS

PATENT NO.: 5,738,990  
ISSUED: April 14, 1998 (19980414)  
INVENTOR(s): Edwards, Cynthia A., Menlo Park, CA (California), US (United States of America)  
Fry, Kirk E., Palo Alto, CA (California), US (United States of America)  
Cantor, Charles R., Boston, MA (Massachusetts), US (United States of America)  
Andrews, Beth M., Maynard, MA (Massachusetts), US (United States of America)  
ASSIGNEE(s): Genelabs Technologies, Inc , (A U.S. Company or Corporation), Redwood City, CA (California), US (United States of America)  
[Assignee Code(s): 33390]  
APPL. NO.: 8-475,221  
FILED: June 07, 1995 (19950607)

This application is a division of application Ser. No. 07-996,783 on Dec. 23, 1992, herein incorporated by reference, which is a continuation-in-part of co-owned U.S. application Ser. No. 07-723,618, filed 27 Jun. 1991 now abandoned.

FULL TEXT: 5857 lines

15/3/37 (Item 33 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02761780

Utility  
ISOLATED NUCLEIC ACID ENCODING RECEPTOR-LIKE TGF-.BETA .1  
BINDING PROTEIN

PATENT NO.: 5,731,200

ISSUED: March 24, 1998 (19980324)  
INVENTOR(s): Ichijo, Hidenori, Uppsala, SE (Sweden)  
Miyazono, Kohei, Uppsala, SE (Sweden)  
Ronnstrand, Lars, Uppsala, SE (Sweden)  
Hellman, Ulf, Uppsala, SE (Sweden)  
Wernstedt, Christer, Uppsala, SE (Sweden)  
Heldin, Carl-Henrik, Uppsala, SE (Sweden)  
ASSIGNEE(s): Ludwig Institute for Cancer Research, (A U.S. Company or  
Corporation), New York, NY (New York), US (United States of  
America)  
[Assignee Code(s): 28349]  
APPL. NO.: 8-567,538  
FILED: December 05, 1995 (19951205)

This application is a divisional of U.S. patent application Ser. No.  
08-167,939, filed as PCT-US92-05199 on Jun. 18, 1992, now U.S. Pat. No.  
5,578,703, which is a continuation in part of application Ser. No.  
07-717,316, filed Jun. 18, 1991, now U.S. Pat. No. 5,229,495.

FULL TEXT: 685 lines

15/3/38 (Item 34 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02758640

Utility

METHOD AND COMPOSITION FOR AN EARLY VACCINE TO PROTECT AGAINST BOTH COMMON  
INFECTIOUS DISEASES AND CHRONIC IMMUNE MEDIATED DISORDERS OR THEIR SEQUELAE

PATENT NO.: 5,728,385  
ISSUED: March 17, 1998 (19980317)  
INVENTOR(s): Classen, John Barthelow, Baltimore, MD (Maryland), US (United  
States of America)  
ASSIGNEE(s): Classen Immunotherapies, Inc , (A U.S. Company or Corporation)  
, Baltimore, MD (Maryland), US (United States of America)  
[Assignee Code(s): 44751]  
APPL. NO.: 8-104,529  
FILED: August 12, 1993 (19930812)  
FULL TEXT: 2719 lines

15/3/39 (Item 35 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02755945

Utility

SCREENING ASSAY FOR THE DETECTION OF DNA-BINDING MOLECULES

PATENT NO.: 5,726,014  
ISSUED: March 10, 1998 (19980310)  
INVENTOR(s): Edwards, Cynthia A., Menlo Park, CA (California), US (United  
States of America)  
Cantor, Charles R., Boston, MA (Massachusettes), US (United  
States of America)  
Andrews, Beth M., Watertown, MA (Massachusettes), US (United  
States of America)  
Turin, Lisa M., Berkeley, CA (California), US (United States  
of America)  
ASSIGNEE(s): Genelabs Technologies, Inc , (A U.S. Company or Corporation),  
Redwood City, CA (California), US (United States of America)  
[Assignee Code(s): 33390]  
APPL. NO.: 8-123,936  
FILED: September 17, 1993 (19930917)

This application is a continuation-in-part of co-owned, co-pending U.S. application Ser. No. 07-996,783, filed 23 Dec. 1992, herein incorporated by reference, which is a continuation-in-part of co-owned, U.S. application Ser. No. 07-723,618, filed 27 Jun. 1991, now abandoned.

FULL TEXT: 13570 lines

15/3/40 (Item 36 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02752915

Utility

METHOD AND COMPOSITION FOR AN EARLY VACCINE TO PROTECT AGAINST BOTH COMMON INFECTIOUS DISEASES AND CHRONIC IMMUNE MEDIATED DISORDERS OR THEIR SEQUELAE

PATENT NO.: 5,723,283  
ISSUED: March 03, 1998 (19980303)  
INVENTOR(s): Classen, John Barthelow, Baltimore, MD (Maryland), US (United States of America)  
ASSIGNEE(s): Classen Immunotherapies, Inc , (A U.S. Company or Corporation) , Baltimore, MD (Maryland), US (United States of America)  
[Assignee Code(s): 44751]  
APPL. NO.: 8-450,586  
FILED: May 31, 1995 (19950531)

This application is a division of PCT-US94-08825, filed Aug. 4, 1994, which is a continuation-in-part of Ser. No. 08-104,529, filed Aug. 12, 1993, now pending. The U.S. national stage of PCT-US94-08825 is Ser. No. 08-591,651, filed Feb. 12, 1996, now pending.

FULL TEXT: 3040 lines

15/3/41 (Item 37 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02748153

Utility

USE OF ENDOGLIN POLYPEPTIDES FOR MODIFYING THE REGULATORY ACTIVITY OF TGF- $\beta$ .

PATENT NO.: 5,719,120  
ISSUED: February 17, 1998 (19980217)  
INVENTOR(s): Letarte, Michelle, Toronto, CA (Canada)  
Massague, Joan, New York, NY (New York), US (United States of America)  
Bernabeu, Carmelo, Madrid, ES (Spain)  
Cheifetz, Sela, Concord, CA (Canada)  
ASSIGNEE(s): HSC Research & Development Limited Partnership, (A Non-U.S. Company or Corporation), CA (Canada)  
[Assignee Code(s): 36023]  
APPL. NO.: 8-459,194  
FILED: June 02, 1995 (19950602)

This application is a continuation of application Ser. No. 07-968,953, filed Oct. 30, 1992.

This invention was made in part with Government support under Grant Nos. CA-08748 and CA-34610, from the National Institutes of Health. The Government may have certain rights in this invention.

FULL TEXT: 877 lines

? t s7/k/1,83,96,103,105,107,110,111

7/K/1 (Item 1 from file: 653)  
DIALOG(R)File 653:(c) format only 1999 Knight-Ridder Info. All rts. reserv.

... class include fusion proteins resulting from insertions at the carboxyl or amino terminal residues of **TGF- beta**. **TGF- beta** fusions with bacterial or other immunogenic proteins are useful for raising **antibodies** against **TGF- beta** or its predetermined fragments.

... the polypeptide in question, or (b) the ability of the candidate polypeptide to compete with **TGF- beta** for a **TGF- beta** cell surface receptor. However, it will be recognized that immunological identity and growth promoting identity are not necessarily coextensive. A neutralizing **antibody** for the mature **TGF- beta** of FIG. 1b may not bind a candidate protein because the neutralizing **antibody** happens to not be directed to a site on **TGF- beta** that is critical for its growth promoting activity. Instead, the **antibody** may bind an innocuous region and exert its neutralizing effect by steric hindrance. Therefore, a candidate protein mutated in this innocuous region might no longer bind the neutralizing **antibody**, but it would nonetheless be **TGF- beta** in terms of substantial homology and biological activity.

The **TGF- beta** residues which are subject to site-directed mutagenesis for the preparation of variants which are...with or without glutaraldehyde cross-linking) for use in the assay or purification of anti-**TGF- beta** **antibodies** or cell surface receptors. **TGF- beta** also is labelled with a detectable group, e.g., radioiodinated by the chloramine T procedure...by EBV immortalization of megakaryoblasts, promegakaryocytes or basophilic megakaryocytes recovered from mammalian bone marrow. The **TGF- beta** of the desired species is recovered from transformant cell cultures by immunoaffinity chromatography using **antibodies** specific for host **TGF- beta**.

Expression vectors for such cells ordinarily include an origin of replication (for extrachromosomal amplification), a promoter located upstream from the **TGF- beta** coding sequences, along with an enhancer if desired, RNA splice site (if intron-containing **TGF**...and preferred, indication is topical application to incisions or exposed tissue for the promotion of wound healing. There are no limitations as to the type of wound or other traumata that can be treated, and these include (but are not limited to): first, second and third degree burns (especially...).

...hydrolysis-resistant **TGF- beta** mutant is employed.

**TGF- beta** also is administered systemically for the treatment of wounds and similar traumata. Systemic administration is useful provided that there are no, or limited, undesirable...

7/K/83 (Item 82 from file: 654)  
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... H<sup>3</sup>thymidine incorporation assay. Each point shows the mean +- standard deviation of triplicate determinations. Anti-**TGF beta** 1 (.circle-solid.), normal rabbit IgG (.largecircle.).

FIGS. 6A and 6B show micrographs demonstrating a decorin-binding cell

regulatory activity that is not suppressed by **antibodies** to TGF **beta -1**.

... morphology of transformed 3T3 cells, and has an activity which is not inhibited with anti-TGF **beta -1 antibody**. Additionally, MRF separates from TGF **beta -1** in HPLC.

The invention still further provides a method of purifying a cell regulatory...

... or reduced. While the method is generally applicable, specific examples of pathologies which can be **treated** include a cancer, a fibrotic disease, and **glomerulonephritis**. In cancer, for example, decorin can be used to bind TGF **beta -1**, destroying TGF...

... of the protein. For example, decorin could be bound by a molecule, such as an **antibody**, which prevents decorin from binding TGF **beta -1**, thus preventing decorin from **inhibiting** the TGF **beta -1** activity. Thus, the TGF **beta -1** wound healing activity could be promoted by binding TGF **beta -1 inhibitors**.

It is understood that modifications which do not substantially affect the activity of the various...largecircle.).

The above result identified the stimulatory factor in the low molecular weight fraction as TGF **beta -1**. However, TGF **beta -1** is not the only active compound in that fraction. Despite the restoration of thymidine incorporation by the anti-TGF **beta -1 antibody** shown in FIG. 5B, the cells treated with the low molecular weight fraction were morphologically different from the cells treated with the control IgG or cells treated with **antibody** alone. This effect was particularly clear when the antibody-treated, low molecular weight fraction was...

7/K/96 (Item 95 from file: 654)  
DIALOG(R)File 654:(c) format only 1999 The Dialog Corp. All rts. reserv.

...patients undergoing radiotherapy or chemotherapy.

The compositions of the present invention may be used for **treating** a wide variety of **wounds** including substantially all cutaneous **wounds**, corneal **wounds**, and injuries to the epithelial-lined hollow organs of the body. **Wounds** suitable for **treatment** include those resulting from trauma such as burns, abrasions, cuts, and the like as well...

...containing compositions in bandages and other wound dressings to provide for continuous exposure of the **wound** to the peptide. Aerosol applications may also find use.

The concentration of polypeptide in the **treatment** composition is not critical. The polypeptide will be present in an epithelial cell proliferation-inducing...Growth of A375 Cells in Nude Mice  
F. Receptor Binding Activity

Example 5: Specificity of **Antibody** to Oncostatin M

- A. Peptide Synthesis
- B. Production of **Antibodies**
- C. Iodination of Oncostatin M

Example 6: Synergistic Effects of Oncostatin M and Other Growth Factors

- A. Oncostatin M, TGF- **beta 1** and rIFN- gamma Effects on A375 Cells
- B. Oncostatin M and TNF- alpha Effects...

... class include fusion proteins resulting from insertions at the carboxyl or amino terminal residues of **TGF- beta**. **TGF- beta** fusions with bacterial or other immunogenic proteins are useful for raising **antibodies** against **TGF- beta** or its predetermined fragments.

... the polypeptide in question, or (b) the ability of the candidate polypeptide to compete with **TGF- beta** for a **TGF- beta** cell surface receptor. However, it will be recognized that immunological identity and growth promoting identity are not necessarily coextensive. A neutralizing **antibody** for the mature **TGF- beta** of FIG. 1b may not bind a candidate protein because the neutralizing **antibody** happens to not be directed to a site on **TGF- beta** that is critical for its growth promoting activity. Instead, the **antibody** may bind an innocuous region and exert its neutralizing effect by steric hindrance. Therefore, a candidate protein mutated in this innocuous region might no longer bind the neutralizing **antibody**, but it would nonetheless be **TGF- beta** in terms of substantial homology and biological activity.

The **TGF- beta** residues which are subject to site-directed mutagenesis for the preparation of variants which are...with or without glutaraldehyde cross-linking) for use in the assay or purification of anti-**TGF- beta** **antibodies** or cell surface receptors. **TGF- beta** also is labelled with a detectable group, e.g., radioiodinated by the chloramine T procedure...by EBV immortalization of megakaryoblasts, promegakaryocytes or basophilic megakaryocytes recovered from mammalian bone marrow. The **TGF- beta** of the desired species is recovered from transformant cell cultures by immunoaffinity chromatography using **antibodies** specific for host **TGF- beta**.

Expression vectors for such cells ordinarily include an origin of replication (for extrachromosomal amplification), a promoter located upstream from the **TGF- beta** coding sequences, along with an enhancer if desired, RNA splice site (if intron-containing **TGF**...and preferred, indication is topical application to incisions or exposed tissue for the promotion of wound healing. There are no limitations as to the type of wound or other traumata that can be treated, and these include (but are not limited to): first, second and third degree burns (especially...

...hydrolysis-resistant **TGF- beta** mutant is employed.

**TGF- beta** also is administered systemically for the treatment of wounds and similar traumata. Systemic administration is useful provided that there are no, or limited, undesirable...

... chimeric transforming growth factor-beta, is biologically active in the standard assay used to measure **TGF- beta** 1 bioactivity and is immunoreactive with **TGF- beta** 1-specific **antibodies**. A chimera structurally comprising a combination of **TGF- beta** 1 and **TGF- beta** 2 amino acid sequences, the **TGF- beta** 1/ beta 2 of the invention is likely to carry a novel portfolio of biological...

... applications include but are not limited to inducing or accelerating cell proliferation and differentiation and, **inhibiting** cell division. Thus **TGF- beta** 1/ beta 2 may find uses in, for example, treating cancer and promoting wound healing.



• The method of the invention may be divided into the following stages solely for... 2-3 week intervals. Bleedings were taken 7-14 days following the boosts.

Anti-peptide **antibodies** directed toward peptide sequences within the **TGF- beta 1** molecule were generated in rabbits using synthetic peptides as immunogens (Gentry et al., 1987, Mol. Cell. Biol. 7:3418-3427). One of the **antibodies** (anti-**TGF- beta 1** sub 369-381) was directed toward epitopes present within the mature form of the **TGF- beta** growth factor. The other two **antibodies** (anti-**TGF- beta 1** sub 81-94 and anti-**TGF- beta 1** sub 225-236) are precursor-specific and are directed toward peptide sequences present only ... CHO-5 **beta 41,2.5** cells secrete approximately 2 mg/L of bioactive chimeric **TGF- beta 1/ beta 2** (FIG. 2).

**TGF- beta** related proteins secreted by these cells were analyzed by immunoblotting using anti-peptide **antibodies** directed against mature **TGF- beta 1** as described in Section 6.1.5., supra FIG. 3 shows that CHO-5...

7/K/107 (Item 106 from file: 654)  
DIALOG(R)File 654:(c) format only 1999 The Dialog Corp. All rts. reserv.

... the polypeptide in question, or (b) the ability of the candidate polypeptide to compete with **TGF- beta** for a **TGF- beta** cell surface receptor. However, it will be recognized that immunological identity and growth promoting identity are not necessarily coextensive. A neutralizing **antibody** for the mature **TGF- beta** of FIG. 1b may not bind a candidate protein because the neutralizing **antibody** happens to not be directed to a site on **TGF- beta** that is critical for its growth promoting activity. Instead, the **antibody** may bind an innocuous region and exert its neutralizing effect by steric hindrance. Therefore, a candidate protein mutated in this innocuous region might no longer bind the neutralizing **antibody**, but it would nonetheless be **TGF- beta** in terms of substantial homology and biological activity.

The **TGF- beta** residues which are subject to site-directed mutagenesis for the preparation of variants which are...with or without glutaraldehyde cross-linking) for use in the assay or purification of anti-**TGF- beta** **antibodies** or cell surface receptors. **TGF- beta** also is labelled with a detectable group, e.g., radioiodinated by the chloramine T procedure...by EBV immortalization of megakaryoblasts, promegakaryocytes or basophilic megakaryocytes recovered from mammalian bone marrow. The **TGF- beta** of the desired species is recovered from transformant cell cultures by immunoaffinity chromatography using **antibodies** specific for host **TGF- beta**.

Expression vectors for such cells ordinarily include an origin of replication (for extrachromosomal amplification), a promoter located upstream from the **TGF- beta** coding sequences, along with an enhancer if desired, RNA splice site (if intron-containing **TGF...**and preferred, indication is topical application to incisions or exposed tissue for the promotion of wound healing. There are no limitations as to the type of wound or other traumata that can be treated, and these include (but are not limited to): first, second and third degree burns (especially...hydrolysis-resistant **TGF- beta** mutant is employed.

**TGF- beta** also is administered systemically for the treatment of wounds and similar traumata. Systemic administration is useful provided that there are no, or limited, undesirable...  
... class include fusion proteins resulting from insertions at the carboxyl or amino terminal residues of **TGF- beta**. **TGF- beta** fusions with bacterial or other immunogenic proteins are useful for raising **antibodies** against **TGF- beta** or its predetermined

fragments.

7/K/110 (Item 109 from file: 654)  
DIALOG(R)File 654:(c) format only 1999 The Dialog Corp. All rts. reserv.

... normal habitants of the digestive tract that invade the bloodstream of patients who receive immunosuppressive **therapy** or suffer from serious underlying trauma or disease, such as severe thermal burns or other serious injuries, cystic **fibrosis**, renal insufficiency, malignant neoplastic diseases, major surgical procedures, or organ transplantations.

The classic septic shock...

...infections has been reduced by using substances such as mafenide acetate or silver salts that **inhibit** bacterial colonization of the burn wound surface and by using potent anti-microbial agents, sympathomimetic amines, corticosteroids, anti-coagulants, granulocyte transfusion, and diuretics for **treating** bacteremia as primary or adjunct therapy.

Such measures, however, have only proved partially successful in...  
... risk with respect to bacteremic Gram-negative infection. Patients at risk include those receiving immunosuppressive **therapy** and those suffering from severe thermal burns or other serious injuries, cystic **fibrosis**, renal failure, or cancer, or are undergoing extensive surgical procedures or organ transplantation. One possible **treatment** is for chronic endobronchitic infection endemic in cystic **fibrosis** patients.

It is within the scope hereof to employ TGF- beta from animals other than ... administration route as other substances such as mafenide acetate, antibiotics, or anti-microbial agents that **inhibit** bacterial colonization of the burn wound surface. Other **therapies** that can be combined with TGF beta **therapy** include primary **therapeutic** agents, for example potent anti-microbial agents such as aminoglycosides (such as amikacin, tobramycin netilmicin...microbial agents of a systemic form, active or passive immunoprophylaxis with typespecific or cross-reactive **antibodies**, and augmentation of the host granulocyte pool with prophylactic granulocyte transfusions.

It is not necessary that such cotreatment drugs be included in the TGF- beta compositions per se although this will be convenient where such drugs are delivered by the...  
... g., antagonists to the activity of TNF- alpha or IL-1, or the abovedescribed neutralizing **antibodies**.

When employed together with the TGF-beta, such agents (other than antibiotics) preferably are employed in lesser dosages than when used alone...is a key mediator of the lethal effects of overwhelming bacterial infection. The administration of **antibodies** to TNF- alpha, in contrast, protects animals from the lethal effects of septic shock. As TGF- beta can decrease endotoxin-stimulated release of TNF- alpha in vitro and in vivo (J. Exo...  
...not yet showing symptoms of septic shock.

6. The method of claim 1 wherein the TGF- beta is administered with a pharmaceutically effective amount of mafenide acetate, an anti-microbial agent, a sympathomimetic amine, an anti-inflammatory agent, a corticosteroid, an anti-coagulant, a diuretic, an **antibody** against tumor necrosis factor, an antibody against lipid A, an antibody against endotoxin core glycolipid...

7/K/111 (Item 110 from file: 654)  
DIALOG(R)File 654:(c) format only 1999 The Dialog Corp. All rts. reserv.

... titer of 1:1,000 on a partially purified CIF-A containing bone extract. The **antibodies** also reacted with **TGF-beta** from platelets, as expected, since the N-terminal sequence is identical.

The competitive ELISA method was used to determine if the **antibodies** detected by the ELISA were antigen-specific **antibodies**. In this experiment, the binding of an optimal dilution of antiserum (1:5,000) to... epithelial layer, intact hair follicles, neovascularization of the graft, and extensive granulation tissue within the wound bed. Few polymorphonuclear cells and mononuclear cells were found within the graft or wound bed, indicating **suppression** of inflammation and graft rejection.

The control skin grafts, pretreated by perfusion with vehicle (1...  
? t s7/3/1,83,96,103,105,107,110,111

7/3/1 (Item 1 from file: 653)  
DIALOG(R)File 653:US Pat.Fulltext  
(c) format only 1999 Knight-Ridder Info. All rts. reserv.

01829702

Utility  
NUCLEIC ACID ENCODING TGF-.BETA. AND ITS USES

PATENT NO.: 4,886,747  
ISSUED: December 12, 1989 (19891212)  
INVENTOR(s): Derynck, Rik M. A., So. San Francisco, CA (California), US  
(United States of America)  
Goeddel, David V., Hillsborough, CA (California), US (United States of America)  
ASSIGNEE(s): Genentech, Inc , (A U.S. Company or Corporation ), South San Francisco, CA (California), US (United States of America)  
[Assignee Code(s): 7579]  
APPL. NO.: 7-25,423  
FILED: March 13, 1987 (13870313)

This is a continuation-in-part of U.S. Ser. No. 715,142 filed Mar. 22, 1985, now abandoned.

FULL TEXT: 1417 lines

7/3/83 (Item 82 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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02598776

Utility  
INHIBITION OF TRANSFORMING GROWTH FACTOR BETA ACTIVITY  
[Binding with decorin]

PATENT NO.: 5,583,103  
ISSUED: December 10, 1996 (19961210)  
INVENTOR(s): Ruoslahti, Erkki I., Rancho Santa Fe, CA (California), US  
(United States of America)  
Yamaguchi, Yu, San Diego, CA (California), US (United States of America)  
ASSIGNEE(s): La Jolla Cancer Research Foundation, (A U.S. Company or Corporation), La Jolla, CA (California), US (United States of America)  
[Assignee Code(s): 9506]  
APPL. NO.: 8-212,311  
FILED: March 14, 1994 (19940314)

This application is a continuation of application Ser. No. 08-050,762,

filed Apr. 20, 1993, now abandoned, which is a continuation of application Ser. No. 07-467,888, filed on Jan. 22, 1990, now abandoned, which is a continuation of application Ser. No. 07-212,702, filed Jun. 28, 1988 now abandoned.

This invention was made with support of government grants CA 30199, CA 42507 and CA 28896 from the National Cancer Institute. Therefore, the United States government may have rights in the invention.

FULL TEXT: 812 lines

7/3/96 (Item 95 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02453615

Utility

ONCOSTATIN M AND NOVEL COMPOSITIONS HAVING ANTI-NEOPLASTIC ACTIVITY  
[DETECTING ONCOSTATIN M RECEPTOR EXPRESSION ON CELLS IN A SAMPLE]

PATENT NO.: 5,451,506  
ISSUED: September 19, 1995 (19950919)  
INVENTOR(s): Shoyab, Mohammed, Seattle, WA (Washington), US (United States of America)  
Zarling, Joyce M., Seattle, WA (Washington), US (United States of America)  
Marquardt, Hans, Mercer Island, WA (Washington), US (United States of America)  
Hanson, Marcia B., Seattle, WA (Washington), US (United States of America)  
Linsley, Peter S., Seattle, WA (Washington), US (United States of America)  
ASSIGNEE(s): Oncogen Limited Partnership, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)  
[Assignee Code(s): 14317]  
APPL. NO.: 8-78,707  
FILED: June 16, 1993 (19930616)

CROSS-REFERENCED RELATED APPLICATIONS

This application is (1) a continuation of Ser. No. 07-397,676 filed Oct. 2, 1989 (abandoned), which is a divisional of Ser. No. 07-144,574, filed Jan. 15, 1988 (abandoned), which is a continuation-in-part of Ser. No. 07-115,139, filed Oct. 30, 1987 (abandoned), and (2) a continuation-in-part of Ser. No. 07-046,846, filed May 4, 1987 (U.S. Pat. No. 5,120,535) the latter being a continuation-in-part of Ser. No. 06,935,283, filed Nov. 26, 1986 (abandoned), which is a continuation-in-part of Ser. No. 06-811,235, filed Dec. 20, 1985 (abandoned), each of which is incorporated herein in its entirety.

FULL TEXT: 1977 lines

7/3/103 (Item 102 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02268540

Utility

NUCLEIC ACID ENCODING TGF-.BETA. AND ITS USES  
[From DNA free of introns; wound healing agents]

PATENT NO.: 5,284,763  
ISSUED: February 08, 1994 (19940208)

- INVENTOR(s): Derynk, Rik M. A., So. San Francisco, CA (California), US  
(United States of America)  
Goeddel, David V., Forestview, CA (California), US (United  
States of America)  
ASSIGNEE(s): Genentech, Inc, (A U.S. Company or Corporation ), South San  
Francisco, CA (California), US (United States of America)  
[Assignee Code(s): 7579]  
APPL. NO.: 7-845,893  
FILED: March 04, 1992 (19920304)  
DISCLAIMER: October 27, 2009 (20091027)

This application is a continuation application of co-pending application  
Ser. No. 07-389,929 filed on 04 Aug. 1989, now U.S. Pat. No. 5,168,051,  
which is a continuation application of U.S. Ser. No. 07-025,423, filed on  
13 Mar. 1987, now U.S. Pat. No. 4,886,747, which is a continuation-in-part  
application of U.S. Ser. No. 06-715,142 filed on 22 Mar. 1985, now  
abandoned.

FULL TEXT: 1404 lines

7/3/105 (Item 104 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02223686

Utility

TGF-.BETA.1/.BETA.2: A NOVEL CHIMERIC TRANSFORMING GROWTH FACTOR-BETA  
[Isolated DNA molecule encoding the factor; mammalian cell transformed with  
DNA molecule coding for factor; producing factor by culturing mammalian  
host cell or transfectant Chinese Hamster Ovary, and recovering factor from  
culture]

PATENT NO.: 5,244,793  
ISSUED: September 14, 1993 (19930914)  
INVENTOR(s): Purchio, Anthony F., Seattle, WA (Washington), US (United  
States of America)  
Madisen, Linda, Seattle, WA (Washington), US (United States of  
America)  
ASSIGNEE(s): Oncogen, (A U.S. Company or Corporation ), Seattle, WA  
(Washington), US (United States of America)  
[Assignee Code(s): 14317]  
APPL. NO.: 7-667,246  
FILED: March 08, 1991 (19910308)

This is a continuation of application Ser. No. 07-284,972, filed Dec. 15,  
1988, now abandoned.

15/3/42 (Item 38 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02745502

Utility  
METHOD OF CONSTRUCTING SEQUENCE-SPECIFIC DNA-BINDING MOLECULES

PATENT NO.: 5,716,780  
ISSUED: February 10, 1998 (19980210)  
INVENTOR(s): Edwards, Cynthia A., Menlo Park, CA (California), US (United States of America)  
Fry, Kirk E., Palo Alto, CA (California), US (United States of America)  
Cantor, Charles R., Boston, MA (Massachusetts), US (United States of America)  
Andrews, Beth M., Watertown, MA (Massachusetts), US (United States of America)  
ASSIGNEE(s): Genelabs Technologies, Inc , (A U.S. Company or Corporation), Redwood City, CA (California), US (United States of America)  
[Assignee Code(s): 33390]  
APPL. NO.: 8-484,499  
FILED: June 07, 1995 (19950607)

This application is a division of application Ser. No. 07-996,783 Dec. 23, 1992, herein incorporated by reference, which is a continuation-in-part of co-owned, U.S. application Ser. No. 07-723,618, filed 27 Jun. 1991 now abandoned.

FULL TEXT: 5477 lines

15/3/43 (Item 39 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02740401

Utility  
METHODS OF INDUCING THE PRODUCTION OF HEMOGLOBIN AND TREATING PATHOLOGIES ASSOCIATED WITH ABNORMAL HEMOGLOBIN ACTIVITY USING PHEMYLACETIC ACIDS AND DERIVATIVES THEROF

PATENT NO.: 5,712,307  
ISSUED: January 27, 1998 (19980127)  
INVENTOR(s): Samid, Dvorit, Rockville, MD (Maryland), US (United States of America)  
ASSIGNEE(s): The United States of America as represented by the Department of Health and Human Services, (A U.S. Government Agency), Washington, DC (District of Columbia, US (United States of America)  
[Assignee Code(s): 6814]  
APPL. NO.: 8-465,924  
FILED: June 06, 1995 (19950606)

This application is a division of applicant's copending U.S. Ser. No. 08-135,661, filed Oct. 12, 1993, which, in turn, is a continuation-in-part of applicant's copending U.S. Ser. No. 07-779,744, filed Oct. 21, 1991, the contents of which are hereby incorporated by reference.

FULL TEXT: 4033 lines

15/3/44 (Item 40 from file: 654)  
DIALOG(R)File 654:US Pat.Full.

(c) format only 1999 The Dialog Corp. All rts. reserv.

02737964

Utility

COMPOSITIONS AND METHODS FOR THERAPY AND PREVENTION OF PATHOLOGIES  
INCLUDING CANCER, AIDS, AND ANEMIA  
[Aryl- and aryloxyalkanoic acids]

PATENT NO.: 5,710,178  
ISSUED: January 20, 1998 (19980120)  
INVENTOR(s): Samid, Dvorit, Rockville, VA (Virginia), US (United States of America)  
ASSIGNEE(s): The United States of America as represented by the Department of Health and Human Services, (A U.S. Government Agency), Washington, DC (District of Columbia, US (United States of America)  
[Assignee Code(s): 6814]  
APPL. NO.: 8-469,691  
FILED: June 06, 1995 (19950606)

This application is a division of Applicant's Ser. No. 08-135,661, filed on Oct. 12, 1993, which, in turn, is a Continuation-In-Part of Applicant's copending U.S. Ser. No. 07-779,744, filed Oct. 21, 1991, the contents of which are hereby incorporated by reference.

FULL TEXT: 4142 lines

15/3/45 (Item 41 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02735492

Utility

METHODS FOR PROMOTING WOUND HEALING  
[ By inducing production of fetal hemoglobins by administering specified aryl acids, derivatives, salts]

PATENT NO.: 5,708,025  
ISSUED: January 13, 1998 (19980113)  
INVENTOR(s): Samid, Dvorit, Rockville, MD (Maryland), US (United States of America)  
ASSIGNEE(s): The United States of America as represented by the Department of Health and Human Services, (A U.S. Government Agency), Washington, DC (District of Columbia, US (United States of America)  
[Assignee Code(s): 6814]  
APPL. NO.: 8-465,835  
FILED: June 06, 1995 (19950606)

This application is a division of Applicant's Ser. No. 08-135,661, filed on Oct. 12, 1993, which in turn, is a Continuation-In-Part of Applicant's copending U.S. Ser. No. 07-779,744, filed Oct. 21, 1991, the contents of which are hereby incorporated by reference.

FULL TEXT: 4036 lines

15/3/46 (Item 42 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02732758

Utility

DECORIN FRAGMENTS INHIBITING CELL REGULATORY FACTORS

PATENT NO.: 5,705,609  
ISSUED: January 06, 1998 (19980106)  
INVENTOR(s): Ruoslahti, Erkki I., Rancho Santa Fe, CA (California), US  
(United States of America)  
Pierschbacher, Michael D., San Diego, CA (California), US  
(United States of America)  
Cardenas, Jose, San Diego, CA (California), US (United States  
of America)  
Craig, William, San Diego, CA (California), US (United States  
of America)  
Mullen, Daniel G., San Diego, CA (California), US (United  
States of America)  
ASSIGNEE(s): La Jolla Cancer Research Foundation, (A U.S. Company or  
Corporation), La Jolla, CA (California), US (United States of  
America)  
[Assignee Code(s): 9506]  
APPL. NO.: 8-442,063  
FILED: May 16, 1995 (19950516)

This application is a continuation of application Ser. No. 07-865,652,  
filed Apr. 3, 1992, now abandoned which is a continuation-in-part of  
application Ser. No. 07-792,192, filed Nov. 14, 1991, now abandoned which  
is a continuation-in-part of Ser. No. 07-467,888, filed Jan. 22, 1990, now  
abandoned which is a continuation-in-part of Ser. No. 07-212,702, filed  
Jun. 28, 1988.

This invention was made with support of government grants CA 30199, CA  
42507 and CA 28896 from the National Cancer Institute. Therefore, the  
United States government may have certain rights in the invention.

FULL TEXT: 2580 lines

15/3/47 (Item 43 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02721620

Utility  
SUPPRESSION OF NITRIC OXIDE PRODUCTION BY OSTEOPONTIN  
[Administering a pentapeptide antiinflammatory agent]

PATENT NO.: 5,695,761  
ISSUED: December 09, 1997 (19971209)  
INVENTOR(s): Denhardt, David T., Bridgewater, NJ (New Jersey), US (United  
States of America)  
Hwang, Shiaw-Min, Piscataway, NJ (New Jersey), US (United  
States of America)  
Heck, Diane Elaine, Rumson, NJ (New Jersey), US (United States  
of America)  
Lopez, Cecilia Ang, North Brunswick, NJ (New Jersey), US  
(United States of America)  
Laskin, Debra L., Basking Ridge, NJ (New Jersey), US (United  
States of America)  
Laskin, Jeffrey D., Piscataway, NJ (New Jersey), US (United  
States of America)  
ASSIGNEE(s): Rutgers University, (A U.S. Company or Corporation),  
Piscataway, NJ (New Jersey), US (United States of America)  
University of Medicine & Dentistry of NJ, (A U.S. Company or  
Corporation), Newark, NJ (New Jersey), US (United States of  
America)  
[Assignee Code(s): 8046; 16819]  
APPL. NO.: 8-173,116  
FILED: December 23, 1993 (19931223)



This research leading to this invention was supported in part by funds from National Institution of Health Grant Nos. AG07972, DC01295, ES047038, GM34310, ES03647 and ES05022. The Government may have certain rights in the invention.

FULL TEXT: 1426 lines

15/3/48 (Item 44 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02719127

Utility  
USES OF TGF-**BETA**. RECEPTOR FRAGMENT AS A THERAPEUTIC AGENT  
[Administering to treat fibroproliferative disorders]

PATENT NO.: 5,693,607  
ISSUED: December 02, 1997 (19971202)  
INVENTOR(s): Segarini, Patricia R., 38 Devonshire Ave., #5, Mountain View, CA (California), US (United States of America), 94043  
Dasch, James R., 837 Seminole, Redwood City, CA (California), US (United States of America), 94062  
Olsen, David R., 276 Hedge Rd., Menlo Park, CA (California), US (United States of America), 94025  
Carrillo, Pedro A., 1966 California St., #7, San Francisco, CA (California), US (United States of America), 94109  
Mascarenhas, Desmond, 1074 Morningside Dr., Sunnyvale, CA (California), US (United States of America), 94087  
[Assignee Code(s): 68000]  
APPL. NO.: 8-361,873

FILED: December 22, 1994 (19941222)  
This application is a continuation of application Ser. No. 08-037,597, filed Mar. 26, 1993, now abandoned which is a continuation-in-part of application Ser. No. 07-968,375, filed Oct. 29, 1992, now abandoned.

FULL TEXT: 1518 lines

15/3/49 (Item 45 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02718988

Utility  
METHOD OF ORDERING SEQUENCE BINDING PREFERENCES OF A DNA-BINDING MOLECULE  
[ Measuring amount of protein bound to double-stranded DNA test oligonucleotide before and after incubation, repeating with different test sequences, comparison for rank order]

PATENT NO.: 5,693,463  
ISSUED: December 02, 1997 (19971202)  
INVENTOR(s): Edwards, Cynthia A., Menlo Park, CA (California), US (United States of America)  
Fry, Kirk E., Palo Alto, CA (California), US (United States of America)  
Cantor, Charles R., Boston, MA (Massachusetts), US (United States of America)  
Andrews, Beth M., Maynard, MA (Massachusetts), US (United States of America), (filed under Rule 47)  
ASSIGNEE(s): Genelabs Technologies, Inc , (A U.S. Company or Corporation), Redwood City, CA (California), US (United States of America)  
[Assignee Code(s): 33390]  
APPL. NO.: 7-996,783  
FILED: December 23, 1992 (19921223)  
DISCLAIMER: April 26, 2011 (20110426)

This application is a continuation-in-part of co-owned, U.S. application Ser. No. 07-723,618, filed 27 Jun. 1991 abandoned.

FULL TEXT: 5514 lines

15/3/50 (Item 46 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02713740

Utility  
OCULAR THERAPY IN SJOGREN'S SYNDROME USING TOPICALLY APPLIED ANDROGENS  
TGF-.BETA.  
[Dry eyes]

PATENT NO.: 5,688,765  
ISSUED: November 18, 1997 (19971118)  
INVENTOR(s): Sullivan, David A., Acton, MA (Massachusetts), US (United States of America)  
ASSIGNEE(s): The Schepens Eye Research Institute, Inc , (A U.S. Company or Corporation), Boston, MA (Massachusetts), US (United States of America)  
[Assignee Code(s): 39547]  
APPL. NO.: 8-477,301  
FILED: June 07, 1995 (19950607)

This application is a continuation-in-part of Sullivan, U.S. patent application Ser. No. 08-124,842, filed Sep. 21, 1993 now U.S. Pat. No. 5,620,921, which was a continuation under 37 CFR 1.62 of Ser. No. 07-871,657, filed Apr. 21, 1992 now abandoned, the whole of which are hereby incorporated by reference herein.

#### GOVERNMENT RIGHTS

Part of the work leading to this invention was made with United States Government funds under Grant No. EY05612 from the National Institutes of Health. Therefore, the U.S. Government has certain rights in this invention.

FULL TEXT: 1271 lines

15/3/51 (Item 47 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02682849

Utility  
METHODS FOR TREATING NEOPLASTIC CONDITIONS USING PHENYLACETIC ACID AND DERIVATIVES THEREOF  
[Administering; treats neuroblastoma, myelodysplasia, non-small cell lung cancer, prostate cancer, melanoma, brain tumor, Kaposi's sarcoma, lymphoma, leukemia, breast and lung cancer, osteosarcoma and fibrosarcoma]

PATENT NO.: 5,661,179  
ISSUED: August 26, 1997 (19970826)  
INVENTOR(s): Samid, Dvorit, Rockville, MD (Maryland), US (United States of America)  
ASSIGNEE(s): The United States of America as represented by the Department of Health and Human Services, (A U.S. Government Agency), Washington, DC (District of Columbia, US (United States of America)  
[Assignee Code(s): 6814]

APPL. NO.: 8-469,466  
FILED: June 06, 1995 (19950606)

This application is a continuation of applicant's Ser. No. 08-135,661, filed Oct. 12, 1993, which, in turn, is a Continuation-In-Part of applicant's U.S. Ser. No. 07-779,744, filed Oct. 21, 1991, and now abandoned the contents of which are hereby incorporated by reference.

FULL TEXT: 3971 lines

15/3/52 (Item 48 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02675188

Utility  
METHODS FOR PREVENTION OF CANCER USING PHENYLACETIC ACIDS AND DERIVATIVES  
THEREOF  
[Therapy for anemia, cancer, aids]

PATENT NO.: 5,654,333  
ISSUED: August 05, 1997 (19970805)  
INVENTOR(s): Samid, Dvorit, Rockville, MD (Maryland), US (United States of America)  
ASSIGNEE(s): The United States of America as represented by the Department of Health and Human Services, (A U.S. Government Agency), Washington, DC (District of Columbia, US (United States of America)  
[Assignee Code(s): 6814]  
APPL. NO.: 8-465,941  
FILED: June 06, 1995 (19950606)

This application is a division of applicant's Ser. No. 08-135,661, filed on Oct. 12, 1993, which, in turn, is a Continuation-In-Part of applicant's copending U.S. Ser. No. 07-779,744, filed Oct. 21, 1991, the contents of which are hereby incorporated by reference.

FULL TEXT: 3978 lines

15/3/53 (Item 49 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02675128

Utility  
USE OF FIBROMODULIN TO PREVENT OR REDUCE DERMAL SCARRING

PATENT NO.: 5,654,270  
ISSUED: August 05, 1997 (19970805)  
INVENTOR(s): Ruoslahti, Erkki I., Rancho Santa Fe, CA (California), US (United States of America)  
Longaker, Michael T., San Francisco, CA (California), US (United States of America)  
Whitby, David J., Adel, GB (United Kingdom)  
ASSIGNEE(s): La Jolla Cancer Research Foundation, (A U.S. Company or Corporation), La Jolla, CA (California), US (United States of America)  
[Assignee Code(s): 9506]  
EXTRA INFO: Assignment transaction [Reassigned], recorded February 17, 1998 (19980217)  
APPL. NO.: 8-303,238  
FILED: September 08, 1994 (19940908)

This application is a continuation of application Ser. No. 07-978,931,

filed Nov. 17, 1992, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-882,345, filed May 13, 1992, now abandoned, which is a continuation of U.S. Ser. No. 07-792,192, filed Nov. 14, 1991, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-467,888, filed Jan. 22, 1990, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-212,702, filed Jun. 28, 1988, now abandoned.

This invention was made with support of government grants CA 30199, CA 42507 and CA 28896 from the National Cancer Institute. Therefore, the United States government may have rights in the invention.

FULL TEXT: 1655 lines

15/3/54 (Item 50 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02658542

Utility  
DIAGNOSIS AND TREATMENT OF CELL PROLIFERATIVE DISEASE HAVING CLONAL  
MACROPHAGE INVOLVEMENT  
[Genetic engineering]

PATENT NO.: 5,639,600  
ISSUED: June 17, 1997 (19970617)  
INVENTOR(s): McGrath, Michael S., Burlingame, CA (California), US (United States of America)  
Herndier, Brian, San Francisco, CA (California), US (United States of America)  
Shiramizu, Bruce, Pacifica, CA (California), US (United States of America)  
ASSIGNEE(s): The Regents of the University of California, (A U.S. Company or Corporation), Oakland, CA (California), US (United States of America)  
[Assignee Code(s): 13234]  
APPL. NO.: 8-473,040  
FILED: June 06, 1995 (19950606)  
CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of our earlier filed U.S. application Ser. No. 08-286,745, filed Aug. 5, 1994 which application is incorporated herein by reference in its entirety and to which application we claim priority under 35 USC selection 120.

#### STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was made at least in part with funds from the Federal government, and the government therefore may have certain rights in the invention.

FULL TEXT: 1626 lines

15/3/55 (Item 51 from file: 654)  
DIALOG(R)File 654:US Pat.Full.

349 479

```

L7      145 PKK223-3
=> s tgf
L8      705 TGF
=> s l8 and extracellular(w)matrix
      4606 EXTRACELLULAR
      119641 MATRIX
      702 EXTRACELLULAR(W)MATRIX
L9      94 L8 AND EXTRACELLULAR(W)MATRIX
=> s l9 and anti(w)tgf(2w)antibod?
      125964 ANTI
      705 TGF
      19115 ANTIBOD?
      12 ANTI(W)TGF(2W)ANTIBOD?
L10     1 L9 AND ANTI(W)TGF(2W)ANTIBOD?
=> d l10 cit,ab

```

1. 5,453,492, Sep. 26, 1995, 60 kDa transforming growth factor-.beta.-binding protein and its use to detect or purify \*\*TGF\*\*-.beta.; Ralf Butzow, et al., 530/413; 435/7.1; 530/350, 395, 402 [IMAGE AVAILABLE]

US PAT NO: 5,453,492 [IMAGE AVAILABLE]

L10: 1 of 1

# ABSTRACT:

This invention provides a novel purified \*\*TGF\*\*-.beta. binding protein, alone or complexed with \*\*TGF\*\*-.beta.. The \*\*TGF\*\*-.beta. binding protein is useful to purify \*\*TGF\*\*-.beta. in a sample and to modify the regulatory activities of \*\*TGF\*\*-.beta..

=> e border, wayne a./in

E#	FILE	FREQUENCY	TERM
E1	USPAT	1	BORDER, ROBERT O/IN
E2	USPAT	1	BORDER, SOLLY/IN
E3	USPAT	0 -->	BORDER, WAYNE A/IN
E4	USPAT	2	BORDERE, LOUIS/IN
E5	USPAT	1	BORDERE, SERGE/IN
E6	USPAT	1	BORDERIE, ANTOINE/IN
E7	USPAT	1	BORDERIES, PIERRE/IN
E8	USPAT	5	BORDERIOU, ARNAUD/IN
E9	USPAT	22	BORDERS, DONALD B/IN
E10	USPAT	6	BORDERS, DONALD BRUCE/IN
E11	USPAT	2	BORDERS, ERNEST D/IN
E12	USPAT	1	BORDERS, FRED C/IN

=> e ruoslahti, erkki/in

E#	FILE	FREQUENCY	TERM
E1	USPAT	2	RUOPSA, JON A/IN
E2	USPAT	1	RUOPSA, JON ARTHUR/IN
E3	USPAT	5 -->	RUOSLAHTI, ERKKI/IN
E4	USPAT	11	RUOSLAHTI, ERKKI I/IN
E5	USPAT	1	RUOSLAHTI, MAIJA/IN
E6	USPAT	1	RUOSS, CHRISTIAN W/IN
E7	USPAT	1	RUOSS, CHRISTIAN WILLIAM/IN
E8	USPAT	2	RUOSS, ERICH/IN
E9	USPAT	2	RUOSS, ERWIN/IN
E10	USPAT	1	RUOSS, HERMANN/IN
E11	USPAT	1	RUOTI, VINCENZO/IN

E12 USPAT 1 RUOTOLO, LISA A/IN

=> s e3,e4

5 "RUOSLAHTI, ERKKI"/IN

11 "RUOSLAHTI, ERKKI I"/IN

L11 16 ("RUOSLAHTI, ERKKI"/IN OR "RUOSLAHTI, ERKKI I"/IN)

=> d l11 1-16cit,ab

'1-16CIT' IS NOT A VALID FORMAT FOR FILE 'USPAT'

ENTER DISPLAY FORMAT (CIT):cit,ab

1. 5,498,694, Mar. 12, 1996, Peptides of the cytoplasmic domain of integrin; \*\*Erkki I. Ruoslahti\*\*, 530/324, 326, 327, 806 [IMAGE AVAILABLE]

US PAT NO: 5,498,694 [IMAGE AVAILABLE]

L11: 1 of 16

ABSTRACT:

Antibodies against integrins are disclosed, as are methods of making same. Such antibodies are elicited by immunizing with synthetic peptides corresponding to the cytoplasmic domains, or functional portions thereof, of various integrin subunits. Peptides employed for such immunization include known cytoplasmic domain amino acid sequences for integrin subunits, as well as newly discovered cytoplasmic domain amino acid sequences for integrin subunits. The latter amino acid sequences are also disclosed and described herein.

=> d l11 2-16 cit,ab

2. 5,453,492, Sep. 26, 1995, 60 kDa transforming growth factor-.beta.-binding protein and its use to detect or purify TGF-.beta.; Ralf Butzow, et al., 530/413; 435/7.1; 530/350, 395, 402 [IMAGE AVAILABLE]

US PAT NO: 5,453,492 [IMAGE AVAILABLE]

L11: 2 of 16

ABSTRACT:

This invention provides a novel purified TGF-.beta. binding protein, alone or complexed with TGF-.beta.. The TGF-.beta. binding protein is useful to purify TGF-.beta. in a sample and to modify the regulatory activities of TGF-.beta..

3. 5,453,489, Sep. 26, 1995, Polypeptide fragments of fibronectin which can modulate extracellular matrix assembly; \*\*Erkki I. Ruoslahti\*\*, et al., 530/350, 324, 395 [IMAGE AVAILABLE]

US PAT NO: 5,453,489 [IMAGE AVAILABLE]

L11: 3 of 16

ABSTRACT:

In accordance with the present invention, there is provided a novel synthetic polypeptide derived from the first type III repeat of fibronectin. The synthetic polypeptide of the invention encompasses a fibronectin-fibronectin binding site, and is capable of inhibiting fibronectin matrix assembly. In contrast to previously identified fibronectin fragments that block fibronectin matrix assembly by blocking an initial event in matrix assembly (i.e., fibronectin binding to cells), the invention polypeptide appears to inhibit an intermediate step in matrix assembly, i.e., fibronectin self-association prior to the disulfide cross-linking that stabilizes the fibronectin matrix.

4. 5,206,347, Apr. 27, 1993, Isolation and use of receptors binding to a

peptide column; \*\*Erkki I. Ruoslahti\*\*, et al., 530/413, 395 [IMAGE AVAILABLE]

US PAT NO: 5,206,347 [IMAGE AVAILABLE]

L11: 4 of 16

ABSTRACT:

A method of isolating cell surface receptors utilizing a short peptide sequence bound to an affinity column. Cell surface receptors which bind selectively to the short peptide and which are specific to various adhesion proteins may be isolated therewith from various cell preparations. These receptors, whose functional integrity has been maintained by the presence of the peptide ligand, are incorporated into liposomes and used to deliver specific compounds inside the liposomes to select tissues containing the specific adhesion proteins.

5. 5,180,809, Jan. 19, 1993, Adhesion receptor for laminin and its use; \*\*Erkki I. Ruoslahti\*\*, et al., 530/350; 424/450; 530/388.22, 389.1, 395, 413, 810 [IMAGE AVAILABLE]

US PAT NO: 5,180,809 [IMAGE AVAILABLE]

L11: 5 of 16

ABSTRACT:

An adhesion receptor for laminin is provided. The receptor is isolated from cell or tissue extracts and fractionated on an affinity column composed of cell attachment-promoting fragments of laminin coupled to Sepharose.TM. in the presence of divalent cations. This receptor can be used to prepare specific antibodies for the analysis of the amount of laminin receptor expressed by cells and has other applications in cellular and tumor biology.

6. 5,180,808, Jan. 19, 1993, Versican core protein, nucleic acid sequences encoding the same, nucleic acid probes, anti-versican antibodies, and methods of detecting the same; \*\*Erkki I. Ruoslahti\*\*, 530/350, 387.9, 388.2, 389.1; 536/23.5 [IMAGE AVAILABLE]

US PAT NO: 5,180,808 [IMAGE AVAILABLE]

L11: 6 of 16

ABSTRACT:

The invention relates to the fibroblast proteoglycan, versican. The versican core protein has the amino acid sequence, and is encoded by the nucleotide sequence, as shown in FIG. 1. The nucleotide sequence and method of hyaluronic acid binding domain is provided and methods of preparing recombinant proteins having hyaluronic acid binding activity are provided. Such protein can be used to determine the presence of hyaluronic acid and as a vehicle to bring other molecules in contact with hyaluronic acid. The complete versican sequence will allow the production of the entire versican molecule to be used, for example, in tissue reconstruction. Nucleic acid probes are provided which are useful for detecting nucleic acid sequences encoding versican. The invention also provides antibodies reactive with versican.

7. 5,169,930, Dec. 8, 1992, Fibronectin receptor; \*\*Erkki I. Ruoslahti\*\*, et al., 530/350, 413 [IMAGE AVAILABLE]

US PAT NO: 5,169,930 [IMAGE AVAILABLE]

L11: 7 of 16

ABSTRACT:

The present invention provides a substantially pure integrin-type

receptor characterized in that it consists of an .alpha..sub.v and a .beta..sub.1 subunit. The .alpha..sub.v .beta..sub.1 integrin binds to fibronectin and GRGDSPK but does not bind to vitronectin. The .alpha..sub.v .beta..sub.1 integrin can be used to determine the presence of a .alpha..sub.v .beta..sub.1 ligand and to develop adhesion peptides specific for the various integrins. The presence of the .alpha..sub.v .beta..sub.1 integrin can be used to assess ability of cells to adhere to fibronectin.

8. 5,041,380, Aug. 20, 1991, Tetrapeptide; \*\*Erkki Ruoslahti\*\*, et al., 435/240.2; 514/2, 18; 530/330 [IMAGE AVAILABLE]

US PAT NO: 5,041,380 [IMAGE AVAILABLE]

L11: 8 of 16

ABSTRACT:

The peptide X-Arg-Gly-Asp-R-Y wherein X is H or at least one amino acid and Y is OH or at least one amino acid, and R is an amino acid selected from Thr or Cys, or other amino acid, having the same cell-attachment activity as fibronectin and the peptide X-Arg-Gly-Asp-Ser-Y, wherein X and Y, having said activity are disclosed.

9. 4,988,621, Jan. 29, 1991, Peptides in cell detachment and aggregation; \*\*Erkki I. Ruoslahti\*\*, et al., 435/240.2, 240.23, 240.243 [IMAGE AVAILABLE]

US PAT NO: 4,988,621 [IMAGE AVAILABLE]

L11: 9 of 16

ABSTRACT:

A method of using synthetic cell attachment-promoting peptides from fibronectin to detach cultured cells from the substratum is described.

10. 4,792,525, Dec. 20, 1988, Tetrapeptide; \*\*Erkki Ruoslahti\*\*, et al., 435/240.243, 180; 530/324, 330, 811, 812, 815; 930/10, 200, 240, DIG.821 [IMAGE AVAILABLE]

US PAT NO: 4,792,525 [IMAGE AVAILABLE]

L11: 10 of 16

ABSTRACT:

The peptide X-Arg-Gly-Asp-R-Y wherein X is H or at least one amino acid and Y is OH or at least one amino acid, and R is an amino acid selected from Thr or Cys, or other amino acid, having the same cell-attachment activity as fibronectin and the peptide X-Arg-Gly-Asp-Ser-Y, wherein X and Y, having said activity are disclosed.

11. 4,661,111, Apr. 28, 1987, Polypeptide; \*\*Erkki I. Ruoslahti\*\*, et al., 623/11; 514/12; 530/350, 395, 813, 815; 623/1, 2, 66; 930/10, DIG.821 [IMAGE AVAILABLE]

US PAT NO: 4,661,111 [IMAGE AVAILABLE]

L11: 11 of 16

ABSTRACT:

A polypeptide having the cell-attaching activity of fibronectin. The polypeptide has 108 amino acid residues and the formula:  
H-Ile-Gly-Gln-Gln-Ser-Thr-Val-Ser-Asp-Val-Pro-Arg-Asp-Leu-Glu-Val-Val-Ala-Ala-Thr-Pro-Thr-Ser-Leu-Leu-Ile-Ser-Trp-Asp-Ala-Pro-Ala-Val-Thr-Val-Arg-Tyr-Tyr-Arg-Ile-Thr-Tyr-Gly-Glu-Thr-Gly-Gly-Asn-Ser-Pro-Val-Gln-Glu-Phe-Thr-Val-Pro-Gly-Ser-Lys-Ser-Thr-Ala-Thr-Ile-Ser-Gly-Leu-Lys-Pro-Gly-Val-Asp-Tyr-Thr-Ile-Thr-Val-Tyr-Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Ala-Ser-



Ser-Lys-Pro-Ile-Ser-Ile-Asn-Tyr-Arg-Thr-Glu-Ile-Asp-Lys-Pro-Ser-Gln-Met-OH. The polypeptide or a biologically active fragment thereof can be employed in the preparation of substrate designed for the attachment of cells thereto. It can be linked to the surface of a prosthetic device to particularly attract endothelial cells and fibroblastic cells.

12. 4,614,517, Sep. 30, 1986, Tetrapeptide; \*\*Erkki Ruoslahti\*\*, et al., 530/330, 300; 604/175; 623/1, 66; 930/10, 200, 240, DIG.821 [IMAGE AVAILABLE]

US PAT NO: 4,614,517 [IMAGE AVAILABLE]

L11: 12 of 16

ABSTRACT:

The peptide X-Arg-Gly-Asp-Ser-Y wherein X is H or at least one amino acid and Y is OH or at least one amino acid having the same cell-attachment activity as fibronectin.

13. 4,589,881, May 20, 1986, Polypeptide; Michael D. Pierschbacher, et al., 623/11; 530/350, 815; 623/66 [IMAGE AVAILABLE]

US PAT NO: 4,589,881 [IMAGE AVAILABLE]

L11: 13 of 16

ABSTRACT:

A polypeptide having the cell-attaching activity of fibronectin. The polypeptide has 108 amino acid residues and the formula:  
H-Ile-Gly-Gln-Gln-Ser-Thr-Val-Ser-Asp-Val-Pro-Arg-Asp-Leu-Glu-Val-Val-Ala-Ala-Thr-Pro-Thr-Ser-Leu-Leu-Ile-Ser-Trp-Asp-Ala-Pro-Ala-Val-Thr-Val-Arg-Tyr-Tyr-Arg-Ile-Thr-Tyr-Gly-Glu-Thr-Gly-Gly-Asn-Ser-Pro-Val-Gln-Glu-Phe-Thr-Val-Pro-Gly-Ser-Lys-Ser-Thr-Ala-T r-Ile-Ser-Gly-Leu-Lys-Pro-Gly-Val-Asp-Tyr-Thr-Ile-Thr-Val-Tyr-Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro-Ile-Ser-Ile-Asn-Tyr-Arg-Thr-Glu-Ile-Asp-Lys-Pro-Ser-Gln-Met-OH. The polypeptide or a biologically active fragment thereof, such as H-Tyr-Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro-Ile-Ser-Ile-Asn-Tyr-Arg-Thr-Glu-Ile-Asp-Lys-Pro-Ser-Gln-Met-OH can be employed in the preparation of substrata designed for the attachment of cells thereto. A Cys-residue may optionally be attached at the C-terminus. It can be linked to the surface of a prosthetic device to particularly attract endothelial cells and fibroblastic cells.

14. 4,578,079, Mar. 25, 1986, Tetrapeptide; \*\*Erkki Ruoslahti\*\*, et al., 623/11; 435/177, 178, 180; 514/2, 802; 530/330; 623/1, 66; 930/10, 200, 220, 240 [IMAGE AVAILABLE]

US PAT NO: 4,578,079 [IMAGE AVAILABLE]

L11: 14 of 16

ABSTRACT:

The peptide X-Arg-Gly-Asp-R-Y wherein X is H or at least one amino acid and Y is OH or at least one amino acid, and R is an amino acid selected from Thr or Cys, or other amino acid, having the same cell-attachment activity as fibronectin and the peptide X-Arg-Gly-Asp-Ser-Y, wherein X and Y, having said activity are disclosed.

15. 4,517,686, May 21, 1985, Polypeptide; \*\*Erkki I. Ruoslahti\*\*, et al., 623/1; 436/501; 514/12; 530/350; 930/10 [IMAGE AVAILABLE]

US PAT NO: 4,517,686 [IMAGE AVAILABLE]

L11: 15 of 16

ABSTRACT:

A polypeptide having the cell-attaching activity of fibronectin. The polypeptide has 108 amino acid residues and the formula:  
H-Ile-Gly-Gln-Gln-Ser-Thr-Val-Ser-Asp-Val-Pro-Arg-Asp-Leu-Glu-Val-Val-Ala-Ala-Thr-Pro-Thr-Ser-Leu-Leu-Ile-Ser-Trp-Asp-Ala-Pro-Ala-Val-Thr-Val-Arg-Tyr-Tyr-Arg-Ile-Thr-Tyr-Gly-Glu-Thr-Gly-Gly-Asn-Ser-Pro-Val-Gln-Glu-Phe-Thr-Val-Pro-Gly-Ser-Lys-Ser-Thr-Ile-Thr-Ile-Ser-Gly-Leu-Lys-Pro-Gly-Val-Asp-Tyr-Thr-Ile-Thr-Val-Tyr-Ala-Val-Thr-Gly-Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro-Ile-Ser-Ile-Asn-Tyr-Arg-Thr-Glu-Ile-Asp-Lys-Pro-Ser-Gln-Met-OH. The polypeptide or a biologically active fragment thereof can be employed in the preparation of substrata designed for the attachment of cells thereto. It can be linked to the surface of a prosthetic device to particularly attract endothelial cells and fibroblastic cells.

16. 4,391,749, Jul. 5, 1983, Method for the purification of collagens; Eva S. Engvall, et al., 530/356, 354, 380, 381, 382, 386 [IMAGE AVAILABLE]

US PAT NO: 4,391,749 [IMAGE AVAILABLE]

L11: 16 of 16

ABSTRACT:

Collagen affinity matrices are prepared, useful for separating collagenous substances from other high molecular weight proteinaceous substances. Fibronectin is fragmentated chemically or by enzymatic digestion, etc. Fragments of fibronectin, which retain the collagen-binding site but are severed from other binding sites of intact fibronectin, are selected and coupled to solid support material.

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...at various stages of amplification will be referred to as TGF beta 1-3-0, TGF beta 1-3-200, TGF beta 1-3-2000 and correspond to CHO cells selected in 0,2 mu M and EXPRESSION OF TGF-beta 1 mRNA IN CHO TRANSFECTANTS

	Number of TGF- beta 1 mRNA Copies Per Cell	sup 1
Cultured	MTX Concentration ( mu M)	
Cells	(0)	(2TGF- beta 1-3 350 34,000 76,000
CHO #7	700	-- --
Non transfected CHO		

20... non-transfected CHO cells and a CHO transfectant (CHO #7) initially shown to express recombinant TGF- beta 1 at low levels. CHO #7 was obtained by cotransfection with pSV2- beta 1-TGF (FIG. 3) and pSV2-neo, followed by selection in Gentamicin (Gibco, N.Y.). The initial TGF- beta 1-3 transfectant displayed mRNA levels of beta 1-TGF similar to those of CHO...

... cells. However, after MTX selection, the number of beta 1-TGF mRNA copies observed in TGF- beta 1-3 cells dramatically increased approaching nearly 80,000 copies per cell at 20 mu M MTX. This represented a greater than 200-fold amplification with respect to the initial TGF- beta 1-3 transfectant and an almost 4000-fold amplification with respect to the non-transfected CHO cells.

A northern blot of poly (A)+ selected mRNA from TGF- beta 1-3 cells at various stages of amplification is shown in FIG. 4A. Non-transfected TGF- beta 1 mRNA present in these cells (asterisk, FIG. 4A). This low level expression of TGF- beta 1 mRNA has also been observed in several other cell lines. The TGF- beta 1-3 transfectants revealed large amounts of hybridizable RNA migrating at the predicted size of 2 Kb.

The most likely mechanism for the dramatic increase of TGF- beta 1 mRNA in the TGF- beta 1-3 cells after MTX selection is a result of amplification. In order to examine for gene amplification, DNA was isolated from the TGF- beta 1-3 cells at various stages of MTX selection, digested with restriction enzymes, transferred to Hybond nylon membranes, and hybridized to [ sup 32 P]-labeled TGF- beta 1 DNA. Both restriction enzymes EcoRI and BamHI cut within the introduced plasmid twice generating...

... plasmid DNA lacking flanking sequences. Since one of the BamHI sites resided within the simian TGF- beta 1 cDNA, digestion with this enzyme should release two fragments which hybridize to the nick... blot are shown in FIG. 4B. In the MTX selected cells, strong hybridization to the TGF- beta 1 probe was observed. The size of these fragments corresponded precisely to the predicted size of the TGF- beta 1 containing fragments of pSV2-TGF- beta 1-dhfr (a 4.7 Kb fragment derived by EcoRI digestion and 4.4 and...

... Similar digestion products were also observed at lower levels in the initial non-selected transfectant (TGF- beta 1 -3/0). Densitometric scans comparing the different TGF- beta 1-3 cells revealed at least a 15- and 35-fold amplification of TGF- beta 1 sequences in TGF- beta 1-3/200 and TGF- beta

1-3/2000 cells, respectively.

### 7.3. DETECTION OF SECRETED BIOACTIVE RECOMBINANT TGF- **beta**.1

To determine whether the TGF- **beta** 1 protein was made and secreted in the transfected TGF- **beta** 1-3 cells, conditioned medium was collected from these cells and tested for bioactive material...

... the initial assay used. A typical standard dose response curve using highly purified authentic human TGF- **beta** 1 is shown in FIG. 5A. In this assay, 50% inhibition of growth of mink lung cells is typically observed at 8-12 picograms (80-120 pg/ml) of TGF- **beta** 1. Conditioned medium collected from TGF- **beta** 1-3 cells at all stages of amplification exhibited the ability to inhibit the growth...

...1-TGF.

Table II shows the levels of bioactive material present in culture supernatants of TGF- **beta** 1-3 cells calculated from the curves shown in FIG. 5B.

TABLE II CONCENTRATION OF BIOLOGICALLY ACTIVE  
TGF- **beta** 1 HARVESTED FROM THE CHO-TRANSFECTANTS sup 1  
Concentration of TGF- **beta** 1 in  
Conditioned Media of CHO Transfectants  
based upon Bioactivity  
Cultured MTX Concentration (  $\mu$  M) TGF- **beta** 1-3 5.8 ng/ml  
1000 ng/ml 5600 ng/ml sup 2

CHO...native TGF to elicit a 50%  
inhibition of CCL64 cells.  
sup 2 Absolute amounts of TGF **beta** produced by the TGF  
**beta** 1-3/200 cel  
line varied from between 1.5 mg/l to 6.5...

...factors including tissue culture conditions of both the indicator cells (CCL64) as well as the TGF **beta** 1-3/200 cells.

Low levels of secreted bioactive TGF- **beta** 1 were observed in supernatants of nonamplified TGF- **beta** 1-3 cells. These levels were similar to the levels observed in the CHO #7 transfectants. MTX selected TGF- **beta** 1-3 cells expressed much higher levels of recombinant bioactive TGF- **beta** 1. At 20  $\mu$  M MTX selection, TGF- **beta** 1-3 cells (TGF- **beta** 1-3/2000) secreted almost 6  $\mu$ g/ml of active TGF- **beta** 1 into the serum free culture supernatants.

Table III shows the levels of bioactive material secreted in culture supernatants of the TGF- **beta** 1-3 transfectant at various stages of MTX selection. Bioactivity was assessed using the mink...

... and the resulting values were normalized per cell per 24 hours. By contrast to the TGF- **beta** 1-3 transfectant, low levels of secreted bioactive TGF- **beta** 1 were observed in the supernatants of the non-amplified TGF- **beta** 1 transfectants. The highest producer of recombinant TGF- **beta** 1 material was the TGF- **beta** 1-3/2000 cells. The amount of bioactive recombinant material secreted by 10 sup 7...

... of tissue culture medium over a period of 24 hours approached nearly 30  $\mu$ g of TGF- **beta** 1. The amount of bioactivity detected in the conditioned supernatants for each of the transfectants correlated with the relative level of TGF- **beta** 1 mRNA observed in these cell lines.

# TABLE III AMOUNT OF BIOACTIVE TGF- $\beta$ 1 SECRETED

BY THE CHO

TRANSFECTANTS AT VARIOUS STAGES OF MTX SELECTION

Transfectant

0 nM 2  $\mu$  M 20  $\mu$  M TGF-  $\beta$  1-3  
 2.9 500 2800 sup b  
 TGF  $\beta$  1 secreted by a confluent 100 mm round tissue culture dish into 5 ml of...  
 ...X  
 10 sup -7 cells) of these cells will secrete nearly 30  $\mu$ g of bioactive TGF  $\beta$  1 into 5 ml of serum free supernatant.

A second bioassay was also employed to further characterize the secreted recombinant TGF-  $\beta$  1. This assay is based on the ability of TGF-  $\beta$  1, in cooperation with EGF-like molecules, to stimulate the growth of NRK cells in...

... The results of this bioassay are shown in Table IV. The activity of the recombinant TGF-  $\beta$  1 is indistinguishable from that of the human natural platelet TGF-  $\beta$  1 used as the control.

## TABLE IV SOFT AGAR COLONY ASSAY COMPARING RECOMBINANT

TGF-  $\beta$  1 AND NATURAL TGF- sup 1  $\beta$  1

Number of Colonies formed  
 in Soft Agar

Recombinant TGF-  $\beta$  1  
 Human

TGF-  $\beta$  1 Collected from

(ng/ml) Conditioned Media Platelet

TGF-  $\beta$  1...growth factor (EGF).

Eight low power fields were counted

per well. No colonies formed when TGF  $\beta$  1 or EGF were absent from the

wells. The amount of recombinant TGF  $\beta$  1 added was estimated based on

the values derived in mink lung epithelial cell inhibition assay. Colony forming activity was assessed using supernatants collected from 20  $\mu$  M MTX selected TGF  $\beta$  1-3 cells.

## 7.4. ACID ACTIVATION OPTIMIZE BIOACTIVITY OF SECRETED RECOMBINANT TGF- $\beta$ 1

Many cell types have been found to secrete natural TGF-  $\beta$  1 in a latent form requiring acidification for optimal bioactivity. The bioassays presented in previous...

... order to determine if the transfected CHO cells secrete a latent biologically inactive form of TGF-  $\beta$  1, serum free supernatants collected from TGF-  $\beta$  1-3/2000 cells were dialyzed against 0.2M acetic acid or 50 mM NH...receiving only the acid dialysis step.

## 7.5. IDENTIFICATION OF MATURE AND PRECURSOR FORMS OF TGF- $\beta$ 1 IN THE CULTURE MEDIUM OF TRANSFECTANT TGF- $\beta$ 1-3 CHO CELLS

Anti-peptide antibodies directed toward peptide sequences within the predicted TGF-  $\beta$  1 molecule were generated in rabbits using synthetic peptides as immunogens. The peptide sequences...

... were utilized are shown in FIG. 7 which also indicates their relative locations within the TGF-  $\beta$  1 precursor polypeptide:

**TGF- beta 1** sub 81-94, **TGF- beta 1** sub 225-236, and **TGF- beta 1** sub 369-381. One of the **antibodies** (anti-**TGF- beta 1** sub 369-381) was directed toward epitopes present within the mature form of the **TGF- beta** growth factor. The other two **antibodies** (anti-**TGF- beta 1** sub 81-94 and anti-**TGF- beta 1** sub 225-236) are precursor-specific and are directed toward peptide sequences present only within the precursor molecule of **TGF- beta 1**.

#### 5 7.5.1. IDENTIFICATION OF MATURE **TGF- beta 1**

Supernatants from the **TGF- beta -3** transfectants were collected and tested by immunoblotting with anti-**TGF- beta 1** sub 369-381 which readily identified authentic mature **TGF- beta 1** (FIG. 8). Specificity was demonstrated by pre-absorbing the **antibody** with synthetic peptide immunogen prior to the immunoblot.

Under reducing conditions (FIG. 8A), authentic **TGF- beta 1** migrates as a polypeptide of 12-13 kd in size. In the supernatants of MTX selected **TGF- beta 1-3** cells, a protein comigrating with authentic **TGF- beta 1** was readily identified (FIG. 8A). Supernatants collected from nonamplified **TGF- beta 1-3** cells displayed nondetectable levels of recombinant **beta 1-TGF** using immunoblotting. The 20  $\mu$  M MTX selected **TGF- beta 1-3** cells appeared to produce the highest levels of mature **TGF- beta 1**, approaching 2-4 times higher than the 2  $\mu$  M MTX selected cells. These...

... Table I) and bioactivity (Table IIA and IIB). Under nonreducing conditions (FIG. 8B), the recombinant **TGF- beta 1** protein behaves as authentic **TGF- beta 1**, migrating as a dimer at 24 kd.

In addition to mature **TGF- beta 1**, larger forms of immunoreactive material were also observed. On reducing gels, these forms ranged...

... The identification of these larger forms as precursor molecules was confirmed using the precursor-specific **antibodies** as described in the next subsection.

#### 7.5.2. IDENTIFICATION OF PRECURSOR **TGF- beta 1**

Supernatants from the **TGF- beta 1-3** transfectants were collected and tested by immunoblotting with the precursor-specific **antibodies** (FIG. 9). After reduction, **antibodies** directed toward two regions of the precursor sequences (anti-**TGF- beta 1** sub 81-94 and anti-**TGF- beta 1** sub 225-236) identified the 44 kd to 56 kd higher molecular weight forms and did not react with the mature **TGF- beta 1** present in the supernatants (FIG. 9A). These precursor-specific **antibodies** in addition to identifying the larger 44 kd to 56 kd forms, also detected ...kd to 42 kd (FIG. 9A). These smaller precursor molecules did not react with anti-**TGF- beta 1** sub 369-381 and may represent only precursor sequences. Thus, supernatants conditioned by **TGF- beta 1-3** cells selected in MTX, contain, in addition to mature **beta 1-TGF**, larger...

... so highly conserved between species, may display other important biological properties. To illustrate all three **TGF- beta 1** forms within the transfectant supernatants after reduction, an immunoblot probed with a mixture of precursor-specific **antibody** (anti-**TGF- beta 1** sub 225-236) and mature **TGF- beta 1** specific **antibody** (anti-**TGF- beta 1** sub 369-381) is shown (FIG. 9A).

Supernatants fractionated on non-reducing SDS-polyacrylamide gels and probed with the precursor-specific peptide **antibodies** or with the mixture of precursor specific **antibody** and mature **TGF- beta 1** specific **antibody** are shown in FIG. 9B. A larger

molecular weight form ranging in size from 95 Kd to 110 Kd was readily identified by each of the antibodies. A mixture of precursor-specific (anti-TGF- $\beta$  1 sub 225-236) and mature TGF- $\beta$  1 (anti-TGF- $\beta$  1 sub 369-381 antibodies detected the dimeric form of TGF- $\beta$  1 (24 Kd) in addition to the 95-110 Kd band.

#### 7.6. RECOMBINANT TGF- $\beta$ 1 CONSTITUTES THE MAJORITY OF THE SECRETED PROTEINS FROM TGF- $\beta$ 1-3-2000 CELLS

TGF- $\beta$  1-3/0 and TGF- $\beta$  1-3/2000 cells were grown to confluency, labeled in serum-free medium with [ sup...

... reducing SDS-polyacrylamide gels. The results are shown in FIG. 10. Supernatants collected from radiolabeled TGF- $\beta$  1-3/0 cells showed no detectable levels of recombinant TGF- $\beta$  1 proteins. In contrast, supernatants from the TGF- $\beta$  1-3/2000 cells revealed four major secreted proteins not found in the initial TGF- $\beta$  1-3/0 transfectant. Three of these proteins migrated identically to the mature and precursor forms of TGF- $\beta$  1 identified by immunoblotting. The other protein which was heavily labeled with [ sup 35 S]-amino acids and released by the TGF- $\beta$  1-3/2000 cells migrates at a molecular weight of 22 Kd. Amino-terminal sequence analysis of this protein revealed its identity as dihydrofolate reductase.

#### 8. EXAMPLE: CHARACTERIZATION OF THE TGF- $\beta$ 1 GENE PRODUCT

The following examples present data on the purification and extensive characterization of...

... The results indicate that rTGF- $\beta$  1 is synthesized in CHO cells as pre-pro-TGF- $\beta$  1 which is processed at the carboxy-terminal side of Gly-29 and Arg 278...

... is not, that rTGF- $\beta$  1 possesses a specific activity equivalent to that of natural TGF- $\beta$  1, and that mature rTGF- $\beta$  1 is a potent inhibitor of tumor cell growth...terminal precursor residues ( b in FIG. 11A).

The cell line used in this example is TGF  $\beta$  3-2000 and the TGF- $\beta$  1 related proteins secreted by these cells, analyzed by immunoblotting, are shown in FIG. 11B. As described in Section 7.5., supra, supernatants derived from TGF  $\beta$  3-2000 cells contain a large 95 Kd-110 Kd form of rTGF- $\beta$  1...

...and a 12 Kd band ( c in FIG. 11B, lane 2) which is the mature TGF- $\beta$  1 monomer. Evidence that bands a, b, and c shown in FIG. 11B contain the...

... easily visualized when [ sup 35 S]-methionine and [ sup 35 S]-cysteine labeled supernatants from TGF- $\beta$  3-2000 cells are analyzed directly by SDS-polyacrylamide gel electrophoresis followed by fluorography (FIG...

... b shown in FIG. 11 suggested that they may be glycosylated. To investigate this possibility, TGF  $\beta$  3-2000 cells were labeled with [ sup 3 H]-glucosamine and cell-free supernatants were...was noted. The same results were obtained using [ sup 35 S]-cysteine labeled supernatants from TGF  $\beta$  3-2000 cells (FIG. 12B). In order to determine the size of the unmodified TGF- $\beta$  1 precursor, a 1350 base pair Pst I-Eco RI fragment containing the entire coding region of TGF- $\beta$  1 (Sharples et al., 1987, DNA 6:239-244) was subcloned into pSP64 and transcribed...that expected for a 390 amino acid protein and most likely corresponds to the unmodified TGF- $\beta$  1 polypeptide backbone. The 39 Kd band shown in FIG. 12A (lane 2) and FIG...

... The band below this corresponds to the deglycosylated band b in FIG. 11A.

Incubation of **TGF beta 3-2000** cells in the presence of [ sup 32 P]-orthophosphate and subsequent fractionation of ACTIVE **TGF-beta.1**

CHO cell transfectants expressing r**TGF-beta 1** (**TGF-beta 3-2000** cells) were propagated and passaged as described in Section 7, supra. Roller bottles...

...M) were seeded with one confluent 150 cm sup 2 round tissue culture dish of **TGF-beta 3-2000** cells and grown at 37 degree(s) C. After cells attached and reached... with r**TGF-beta 1** using the same column conditions. The low apparent molecular weight of **TGF-beta 1**, as determined by gel permeation chromatography, may be due to the tightly folded structure...determined.

The purified polypeptide was chemically cleaved with cyanogen bromide prior to sequencing. Since mature **TGF-beta 1** contains only one methionine at residue 382, two sequences were obtained simultaneously; one corresponding... **beta 1** (FIG. 16). Also apparent on the gels were the two precursor species, pro-**TGF-beta 1** (30-390) and the 30-42 KDa pro region of the precursor (30-278...

... precursor purified from the conditioned medium of the CHO cells represents a mixture of pro-**TGF-beta 1** (30-390), the pro region of the precursor (30-278), and the mature chain...

... with CNBr and purified the CNBr peptides to establish the chemical nature of this complex. **TGF-beta 1**-precursor (800 pmol) was dissolved in 30 mu l of 70% formic acid and... peptide M(30-38/262-382/383-390) represents a disulfide-linked peptide involving pro-**TGF-beta 1**.

TABLE VII...et al., 1987, J. Biol. Chem. 262:12127-12136).

Serum-free conditioned media from CHO-**TGF-beta 1-3/2000** cells expressing high levels of simian r**TGF-beta 1** was electrophoresed on 44-56 KDa ("a" in FIG. 17) and possessing immunological epitopes derived from **TGF-beta 1** precursor and mature **TGF-beta 1** (Section 7.5, supra), most likely represents unprocessed **TGF-beta 1** precursor. The 30-42 KDa polypeptide ("b" in FIG. 17) only contains precursor-derived...

... 5, supra) indicating that this species has undergone proteolytic cleavage separating it and the mature **TGF-beta 1** form. The 14 KDa species ("c" in FIG. 17) represents the mature, fully processed **TGF-beta 1** monomer. Recombinant precursor proteins ("a" and "b" in FIG. 17) were electroeluted from acrylamide...

... have identical amino-terminal sequences. Comparison of this sequence with that predicted from the simian **TGF-beta 1** cDNA (Sharples, et al., 1987, DNA 6:239-244) indicates that both larger proteins...

...Gly-29/Leu-30 removing the first 29-amino acids of the intact pre-pro-**TGF-beta 1** molecule. Cleavage of this hydrophobic 29-amino acid leader sequence is most likely the... 1986, Nucleic Acids Res. 14:4683-4690). Based on these results, the 44-56 KDa **TGF-beta 1** polypeptide ("a" in FIG. 17) represents pro-**TGF-beta 1** (30-390) whereas the 30-42 KDa species ("b" in FIG. 17) corresponds to the pro region of the precursor (30-278) lacking the signal peptide and mature **TGF-beta 1** sequences. Sequence analysis of the 14 KDa polypeptide (data not shown) revealed an intact amino-terminus beginning at Ala-279 of the mature growth factor indicating that CHO-**TGF-beta 1-3/2000** cells properly process the simian r**TGF-beta 1** at the dibasic cleavage site. A summary of a proposed processing scheme for **TGF-beta 1** is presented in FIG. 20.



## 8.5. BIOLOGICAL ACTIVITY IN VITRO

Purified mature and...

... 6., supra. The biological activity profiles of mature and precursor rTGF-  $\beta$  1 and natural TGF-  $\beta$  1 from bovine spleen are presented in FIG. 19. Molarity calculations were based on predicted amino acid compositions (for precursor rTGF-  $\beta$  1, the amino acid composition of pro-TGF-  $\beta$  1 was used). The results indicate that mature rTGF-  $\beta$  1 is a potent inhibitor...

... maximal inhibition) and has an activity curve which is superimposable with that obtained for natural TGF-  $\beta$  1. In other words, the recombinant TGF-  $\beta$  1 of the invention and natural TGF-  $\beta$  1 possess identical specific activities. In contrast, the precursor preparation was 50-fold less active...

...between the mature and precursor forms.

## 8.6. BIOLOGICAL ACTIVITY IN VIVO

The effects of TGF-  $\beta$  1 in vivo are largely unknown although its role in inhibiting mammary gland growth (Silberstein...

... embryonic development (Weeks et al., 1987, Cell 51:861) have been suggested. Both naturally derived TGF-  $\beta$  1 and TGF-  $\beta$  2 from bone (Seyedin et al., ...Chem. 261:5693; Seyedin et al., 1987, J. Biol. Chem. 262:1946) and recombinant Simian TGF-  $\beta$  1 cloned (Sharples et al., 1987, DNA 6:239-244) and expressed (Gentry et al., 1987, Mol. Cell. Biol. 7:3418) in CHO-TGF-  $\beta$  1-3-2000 cells are potent inhibitors of DNA synthesis in a variety of established...

...Biochem. Biophys. Res. Comm. 148:783). In this study, we present in vivo evidence that TGF-  $\beta$  is also tumorstatic for a human lung tumor grown in athymic nude mice. Particularly striking is the induction by TGF-  $\beta$  of a differentiated-like cellular phenotype in the inhibited tumor cells. The human lung carcinoma...

... the lung, is a responsive target (in vitro) for inhibition by picomolar concentrations of natural TGF-  $\beta$  1 or TGF-  $\beta$  2 and the recombinant TGF-  $\beta$  1 of the invention. Nude mice were subcutaneously inoculated with A549 cells; palpable tumors developed in approximately 80% of the animals in three weeks. FIG. 21 shows the effect of TGF-  $\beta$  1 and TGF-  $\beta$  2 treatment on the further growth of A549 tumors in these mice. Each experimental group...

... in control groups received injections of bovine serum albumin in a carrier solution identical to TGF-  $\beta$  treated tumor-bearing animal groups. Tumor volume in control groups doubled approximately every 7-8...

... successive injections, corresponding to those days as indicated on the abscissa, of 200 ng of TGF-  $\beta$  1 and TGF-  $\beta$  2 (1.4  $\mu$ g total per treatment regimen for each) were tumorstatic and retarded the further growth of tumors. As shown in FIG. 21, TGF-  $\beta$  1 appeared slightly more effective than TGF-  $\beta$  2.

In a separate experiment, the dose response of TGF-  $\beta$  1 on A549 tumor inhibition was examined (FIG. 21). Values represent average tumor volumes in TGF-  $\beta$  treated animals relative to tumor volumes from mock-treated animals. About 25% inhibition was observed at the lowest dose tested (12.5 ng per injection) of TGF-  $\beta$  1. At higher doses, 50 and 200 ng per injection, a correspondingly greater inhibition in...

...volume relative to tumors derived from control groups. Animals receiving even the highest doses of TGF-  $\beta$  (1.4  $\mu$ g total after 20

days) exhibited no gross manifestations of TGF-  $\beta$  toxicity. As shown in FIG. 23, TGF-  $\beta$  1 treated animals displayed normal characteristics; no apparent abnormalities were found on gross examination of...

...21, FIG. 21) had a greater than 90% reduction in average net weights.

Recombinant Simian TGF-  $\beta$  1 purified to homogeneity from serum-free culture supernatants exhibits an in vitro dose response...tumor cells used in these studies. A comparison of the effect of recombinant and natural TGF-  $\beta$  1 on the growth of A549 human lung carcinoma in athymic mice is shown in Table VIII. The recombinant product was more effective in inhibiting tumor growth than bone-derived TGF-  $\beta$  1, 60% inhibition compared to 46% inhibition.

TABLE VIII...

What is claimed is:

1. A recombinant simian TGF-  $\beta$  1 polypeptide comprising an amino acid sequence as depicted in FIG. 1.
2. A recombinant simian TGF-  $\beta$  1 polypeptide of claim 1 comprising amino acid residue numbers 1 to 390.
3. A recombinant simian TGF-  $\beta$  1 polypeptide of claim 1 comprising amino acid residue numbers 30 to 390.
4. A recombinant simian TGF-  $\beta$  1 polypeptide of claim 1 comprising amino acid residue numbers 30 to 278.
5. A recombinant simian TGF-  $\beta$  1 polypeptide of claim 1, 2, 3 or 4 which is glycosylated.
6. A recombinant simian TGF-  $\beta$  1 polypeptide of claim 1, 2, 3 or 4 which is phosphorylated.
7. A recombinant simian TGF-  $\beta$  1 polypeptide of claim 6 which contains at least one mannose-6-phosphate.
8. A recombinant simian TGF-  $\beta$  1 polypeptide of claim 26 containing at least one mannose-6-phosphate linked to an asparagine linked sugar chain of the rTGF-  $\beta$  1 precursor.
9. A recombinant simian TGF-  $\beta$  1 polypeptide of claim 6 containing an asparagine at amino acid residue number 82.
10. A recombinant simian TGF-  $\beta$  1 polypeptide of claim 6 containing an asparagine at amino acid residue number 136.
11. A recombinant simian TGF-  $\beta$  1 polypeptide of claim 6 containing an asparagine at amino acid residue number 176.
12. A recombinant simian TGF-  $\beta$  1 polypeptide comprising an amino acid sequence as depicted in FIG. 1 in which amino acid residue number 33 is serine.
13. A recombinant simian TGF-  $\beta$  1 polypeptide comprising an amino acid sequence as depicted in FIG. 1 in which amino acid residue number 223 is serine.
14. A recombinant simian TGF-  $\beta$  1 polypeptide comprising an amino acid sequence as depicted in FIG. 1 in which amino acid residue number 225 is serine.
15. A recombinant simian TGF-  $\beta$  1 polypeptide comprising an amino acid sequence as depicted in FIG. 1 in which amino acid residue numbers 223 and 225 are serine.

16. A recombinant simian **TGF- beta 1** polypeptide of claim 12, 13, 14, or 15 comprising amino acid residue numbers 1 to 390.
17. A recombinant simian **TGF- beta 1** polypeptide of claim 12, 13, 14 or 15 comprising amino acid residue numbers 30 to 390.
18. A recombinant simian **TGF- beta 1** polypeptide of claim 12, 13, 14, or 15 comprising amino acid residue numbers 30 to 278.
19. A recombinant simian **TGF- beta 1** polypeptide of claim 12, 13, 14, or 15, which is glycosylated.
20. A recombinant simian **TGF- beta 1** polypeptide of claim 12, 13, 14, or 15, which is phosphorylated.
21. A recombinant simian **TGF- beta 1** polypeptide of claim 20 which contains at least one mannose-6-phosphate.
22. A recombinant simian **TGF- beta 1** polypeptide of claim 21 in which the mannose-6-phosphate linked to an asparagine linked sugar chain of the r**TGF- beta 1** precursor.
23. A recombinant simian **TGF- beta 1** polypeptide of claim 20 containing an asparagine at amino acid residue number 82.
24. A recombinant simian **TGF- beta 1** polypeptide of claim 20 containing an asparagine at amino acid residue number 136.
25. A recombinant simian **TGF- beta 1** polypeptide of claim 20 containing an asparagine at amino acid residue number 176.
26. The recombinant simian **TGF- beta 1** polypeptide of claim 16 which is glycosylated.
27. The recombinant simian **TGF- beta 1** polypeptide of claim 17 which is glycosylated.
28. The recombinant simian **TGF- beta 1** polypeptide of claim 18 which is glycosylated.
29. The recombinant simian **TGF- beta 1** polypeptide of claim 16 which is phosphorylated.
30. The recombinant simian **TGF- beta 1** polypeptide of claim 17 which is phosphorylated.
31. The recombinant simian **TGF- beta 1** polypeptide of claim 18 which is phosphorylated.
32. An isolated latent simian recombinant **TGF- beta 1** gives rise to biologically active **TGF- beta 1** upon acid activation.

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#### ABSTRACT

... chain, and more specifically to active fragments of decorin or its functional equivalents to bind **TGF beta**. The invention also provides a novel cell regulatory factor designated MRF. Also provided are methods...  
...3 for molecular weight standards.

FIGS. 2A and 2B show binding of [ <sup>125</sup> I]**TGF beta 1** to decorin-Sepharose: (FIG. 2A) Fractionation of [ <sup>125</sup> I]-**TGF**

**beta 1** to decorin-Sepharose affinity chromatography. [ sup 125 I] **TGF beta 1** (5X10 sup 5 cpm) was incubated in BSA-coated polypropylene tubes with 0.2...by SDS-polyacrylamide gel under nonreducing conditions. Lane 1: the original [ sup 125 I]-labeled **TGF beta 1** sample; lanes 2-7: flow through and wash fractions; lanes 8-10: 3M NaCl ...  
...separating gel.

FIGS. 3A and 3B show the inhibition of binding of [ sup 125 I]**TGF beta 1** to decorin by proteoglycans and their core proteins: (FIG. 3A) Competition of [ sup 125 I]**TGF beta 1** binding to decorin-coated microtiter wells by recombinant decorin (.circle-solid.), decorin isolated from...

... Each point represents the mean of duplicate determinants. (FIG 3B) Competition of [ sup 125 I]**TGF beta 1** binding with chondroitinase ABC-treated proteoglycans and BSA. The concentrations of competitors ...in FIG. 3A.

FIGS. 4A and 4B show neutralization of the growth regulating activity of **TGF beta 1** by decorin: (FIG. 4A) Shows inhibition of **TGF beta 1**-induced proliferation of CHO cells by decorin. [ sup 3 H]Thymidine incorporation assay was performed in the presence of 5 ng/ml of **TGF beta -1** and the indicated concentrations of purified decorin (.largecircle.) or BSA (.circle-solid.). At the concentration used, **TGF beta -1** induced a 50% increase of [ sup 3 H]thymidine incorporation in the CHO cells...

... this growth stimulation; i.e. [ sup 3 H]thymidine incorporation in the absence of either **TGF beta 1** or decorin=0%, incorporation in the presence of **TGF beta** but not decorin=100%. Each point shows the mean +- standard deviation of triplicate samples. (FIG. 4B) Shows neutralization of **TGF beta 1**-induced growth inhibition in Mv1Lu cells by decorin. The assay was performed as in A except that **TGF beta -1** was added at 0.5 ng/ml. This concentration of **TGF beta -1** induces 50% reduction of [ sup 3 H]thymidine incorporation in the Mv1Lu cells. The data represent neutralization of **TGF beta -1**-induced growth inhibition; i.e. [ sup 3 H]thymidine incorporation in the presence of neither **TGF beta** or decorin=100%; incorporation in the presence of **TGF beta** but not decorin=0%.

FIG. 5A shows separation of growth inhibitory activity from decorin-expressing...

...Cy:cytochrome c (Mr=12,400); Ap:aprotinin (Mr=6,500); TGF: [ sup 125 I] **TGF beta 1** (Mr=25,000).

FIG. 5B shows identification of the growth stimulatory material from gel filtration as **TGF beta 1**. The growth stimulatory activity from the late fractions from Sepharose 6B (bar in panel A) was identified by inhibiting the activity with protein A-purified IgG from an anti-**TGF beta** antiserum. Data represent percent inhibition of growth stimulatory activity in a [ sup 3 H]thymidine incorporation assay. Each point shows the mean +- standard deviation of triplicate determinations. Anti-**TGF beta 1** (.largecircle.), normal rabbit IgG (.circle-solid.).

FIG. 6 is a schematic diagram of MBP...

...maltose binding protein.

FIG. 7 shows the results of binding studies of sup 125 I-**TGF beta** to immobilized recombinant decorin (DC13) and MBP-decorin fragments PT-65, PT-71, PT-72 and PT-73.

FIG. 8 shows the results of binding studies of sup 125 I-**TGF**

**beta** to immobilized decorin (DC-18v) and MBP-decorin fragments PT-71, PT-72, PT-84...

...and PT-87.

FIG. 9 shows the results of binding studies of sup 125 I-TGF **beta** 1 to HepG2 cells in the presence of decorin fragments PT-65, PT-71, PT...

...and PT-78.

FIG. 10 shows the results of binding studies of sup 125 I-TGF **beta** to L-M(tk-) cells in the presence of decorin and decorin fragments PT-71 PT-85.

FIG. 11 shows the results of binding studies of sup 125 I-TGF **beta** 1 to L-M(tk-) cells in the presence of decorin and recombinant decorin fragments...

...and PT-87.

FIG. 12 shows the results of binding studies of sup 125 I-TGF **beta** 1 to L-M(tk-) cells in the presence of synthetic decorin peptide fragments P...

...to the N-terminal 15-mer.

FIG. 13 shows the results of sup 125 I-TGF **beta** binding to immobilized decorin with or without the presence of synthetic decorin peptide fragments 16D...  
...4:229, (1988); McFarland et al., Science 245:494 (1989)).

Transforming growth factor beta's (TGF **beta**) are a family of multi-functional cell regulatory factors produced in various forms by many ...

... cells (for review see Sporn et al., J. Cell Biol. 105:1039, (1987)). Five different TGF **beta**'s are known, but the functions of only two, TGF **beta** -1 and TGF **beta** -2, have been characterized in any detail. TGF **beta**'s are the subject of U.S. Pat. Nos. 4,863,899; 4,816,561; and 4,742,003 which are incorporated by reference. TGF **beta** -1 and TGF **beta** -2 are publicly available through many commercial sources (e.g. R & D Systems, Inc., Minneapolis, Minn.). These two proteins have similar functions and will be here collectively referred to as TGF **beta**. TGF **beta** binds to cell surface receptors possessed by essentially all types of cells, causing profound changes in them. In some cells, TGF **beta** promotes cell proliferation, in others it suppresses proliferation. A marked effect of TGF **beta** is that it promotes the production of extracellular matrix proteins and their receptors by cells (for review see Keski-Oja et al., J. Cell...  
... Roberts and Sporn in "Peptides Growth Factors and Their Receptors" (Springer-Verlag, Heidelberg (1989)).

While TGF **beta** has many essential cell regulatory functions, improper TGF **beta** activity can be detrimental to an organism. Since the growth of mesenchyme and proliferation of mesenchymal cells is stimulated by TGF **beta**, some tumor cells may use TGF **beta** as an autocrine growth factor. Therefore, if the growth factor activity of TGF **beta** could be prevented, tumor growth could be controlled. In other cases the inhibition of cell proliferation by TGF **beta** may be detrimental, in that it may prevent healing of injured tissues. The stimulation of extracellular matrix production by TGF **beta** is important in situations such as wound healing. However, in some cases the body takes...

...of excessive accumulation of extracellular matrix is glomerulonephritis, a disease with a detrimental involvement of **TGF beta**.

Thus, a need exists to develop compounds that can modulate the effects of cell regulatory factors such as **TGF beta**. The present invention satisfies this need and provides related advantages.

#### SUMMARY OF THE INVENTION

The fragments of decorin or a functional equivalent of decorin to bind **TGF beta** or other cell regulatory factors. The invention also provides a novel cell regulatory factor designated...

... cell surface receptors and include growth factors. Examples of cell regulatory factors include the five **TGF beta**'s, platelet-derived growth factor (PDGF), epidermal growth factor, insulin like growth factor I and...

... be bound by different proteins which can affect the regulatory factor's activity. For example, **TGF beta** -1 is bound by decorin and biglycan, and MRF by decorin.

By "cell regulatory factor ... be recognized by their ability to competitively inhibit the binding of, for example, decorin to **TGF beta**, or of other polypeptides to their cognate growth factors.

Active fragments can be obtained by **TGF beta** and an active fragment can then be contacted with the affinity matrix and the amount of **TGF beta** bound thereto determined.

As used herein, "decorin" refers to a proteoglycan having substantially the structural... a cell regulatory factor. Specifically, the invention teaches the discovery that decorin and biglycan bind **TGF beta** -1 and MRF and that such binding can inhibit the cell regulatory functions of **TGF beta** -1. Further, both decorin and biglycan are about 80% homologous and contain a leucine-rich...microtiter assay as set forth in Example II, using known cell regulatory factors, such as **TGF beta** -1. Alternatively, any later discovered cell regulatory factor could be tested, for example by affinity...

... Moreover, one skilled in the art could simply substitute a novel cell regulatory factor for **TGF beta** -1 or a novel leucine-rich repeat protein for decorin or biglycan in the Examples...morphology of transformed 3T3 cells, and has an activity which is not inhibited with anti-**TGF beta** -1 antibody. Additionally, MRF separates from **TGF beta** -1 in HPLC.

The invention still further provides a method of purifying a cell regulatory...

...which becomes bound to the protein. The method can be used, for example, to purify **TGF beta** -1 by using decorin.

The invention additionally provides a method of treating a pathology caused by a **TGF beta** -regulated activity comprising contacting the **TGF beta** with a purified polypeptide, wherein the polypeptide comprises the **TGF beta** binding domain of a protein and wherein the protein is characterized by a leucine-rich...

... or reduced. While the method is generally applicable, specific examples of pathologies which can be treated include a cancer, a fibrotic disease, and glomerulonephritis. In cancer, for example, decorin can be used to bind **TGF beta** -1, destroying **TGF beta** -1's growth stimulating activity on the cancer cell.

Finally, a method of preventing the...

... of the protein. For example, decorin could be bound by a molecule, such

as an **antibody**, which prevents decorin from binding **TGF beta -1**, thus preventing decorin from inhibiting the **TGF beta -1** activity. Thus, the **TGF beta -1** wound healing activity could be promoted by binding **TGF beta -1** inhibitors.

It is understood ...which do not substantially affect the activity of the various molecules of this invention including **TGF beta**, MRF, decorin, biglycan and fibromodulin are also included within the definition of those molecules. It...SDS-polyacrylamide electrophoresis are collected and represent purified decorin core protein.

#### EXAMPLE II

#### BINDING OF **TGF.beta.** TO DECORIN

##### A. Affinity Chromatography of **TGF beta** on Decorin-Sepharose

Decorin and gelatin were coupled to cyanogen bromide-activated Sepharose (Sigma) by...

... of protein per ml of Sepharose matrix according to the manufacturer's instructions. Commercially obtained **TGF beta -1** (Calbiochem, La Jolla, Calif.) was sup 125 I-labelled by the chloramine T method...

... Biol. Chem. 259:10995-11000 (1984)) which is incorporated herein by reference and the labeled **TGF beta** was separated from the unreacted iodine by gel filtration on Sephadex G-25, equilibrated with...

... buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) (FIG. 2). [ sup 125 I]-**TGF beta 1** (5X10 sup 5 cpm) was incubated in BSA- ...columns and the SDS-PAGE analysis of the fractions is shown in FIG. 2B. The **TGF beta -1** starting material contains a major band at 25 kd. This band represents the native **TGF beta -1** dimer. In addition, there are numerous minor bands in the preparation. About 20-30 components and some of the 25 kd **TGF beta -1**, whereas the bound, urea-eluted fraction contains only **TGF beta -1** (FIG. 2B). These results show that **TGF beta -1** binds specifically to decorin, since among the various components present in the original **TGF beta -1** preparation, only **TGF beta -1** bound to the decorin-Sepharose affinity matrix and since there was very little binding to the control gelatin-Sepharose affinity matrix. The **TGF beta -1** that did not bind to the decorin-Sepharose column may have been denatured by the iodination. Evidence for this possibility was provided by affinity chromatography of unlabeled **TGF beta -1** as described below.

In a second experiment, unlabeled **TGF beta -1** 180 ng was fractionated on decorin-Sepharose as described above for sup 125 I-**TGF beta**.

**TGF beta -1** (180 ng) was incubated with decorin-Sepharose or BSA-agarose (0.2 ml packed... inhibition of [ sup 3 H]thymidine incorporation in Mv1Lu cells (Example III). The amounts of **TGF beta -1** in each pool were calculated from the standard curve of [ sup 3 H]thymidine incorporation obtained from a parallel experiment with known concentration of **TGF beta -1**. The results show that the **TGF beta -1** bound essentially quantitatively to the decorin column, whereas there was little binding to the control column (Table 1). The partial recovery of the **TGF beta -1** activity may be due to loss of **TGF beta -1** in the dialyses.

TABLE I Decorin-Sepharose affinity chromatography of nonlabeled **TGF beta -1** monitored by growth inhibition assay in Mv1Lu cells.

Elution	<b>TGF beta -1</b> (ng)	Decorin-Sepharose	BSA-Sepharose	B. Binding of <b>TGF</b>
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**beta -1** to Decorin in a Microtiter Assay: Inhibition by Core Protein and Biglycan

The binding of **TGF beta -1** to decorin was also examined in a microtiter binding assay. To perform the assay...

...05% Tween (PBS/Tween) and samples containing 5X10<sup>4</sup> sup 4 cpm of [ sup 125 I]-**TGF beta -1** and various concentrations of competitors in PBS/Tween were added to each well. The... under the conditions used. Nonspecific binding, determined by adding 100-fold molar excess of unlabeled **TGF beta -1** over the labeled **TGF beta -1** to the incubation mixture, was about 13% of total binding. This assay was also... decorin and decorin isolated from bovine skin (PGII) inhibited the binding of [ sup 125 I]-**TGF beta -1**, as expected (FIG. 3A). Biglycan isolated from bovine articular cartilage was as effective an...

... chondroitinase ABC did not shown any inhibition. Additional binding experiments showed that [ sup 125 I]-**TGF beta -1** bound to microtiter wells coated with biglycan or its chondroitinase-treated core protein. These results show that **TGF beta -1** binds to the core protein of decorin and biglycan and implicates the leucine-rich...

#### ...EXAMPLE III

##### ANALYSIS OF THE EFFECT OF DECORIN ON CELL PROLIFERATION STIMULATED OR INHIBITED BY **TGF.beta.-1**

The ability of decorin to modulate the activity of **TGF beta -1** was examined in [ sup 3 H]thymidine incorporation assays. In one assay, an unamplified...

... H]thymidine incorporation was assayed as described (Cheifetz et al., Cell 48:409-415 (1987)). **TGF beta -1** was added to the CHO cell cultures at 5 ng/ml. At this concentration percent neutralization of the **TGF beta -1**-induced growth stimulation, i.e., [ sup 3 H]thymidine incorporation, in the absence of either **TGF beta -1** or decorin=0%, incorporation in the presence of **TGF beta -1** but not decorin=100%. Each point shows the mean  $\pm$  standard deviation of triplicate samples. Decorin (.circle-solid.) BSA (.largecircle.).

Decorin neutralized the growth stimulatory activity of **TGF beta -1** with a half maximal activity at about 5  $\mu$ g/ml. Moreover, additional decorin suppressed the [ sup 3 H]-thymidine incorporation below the level observed without any added **TGF beta -1**, demonstrating that decorin also inhibited **TGF beta** made by the CHO cells themselves. Both the decorin-expressor and control CHO cells produced an apparently active **TGF beta** concentration of about 0.25 ng/ml concentration into their conditioned media as determined by...

... interference from the decorin in the culture media because, as shown below, the effect of **TGF beta** on the mink cells was not substantially inhibited at the decorin concentrations present in the CCL64) also revealed an effect by decorin on the activity of **TGF beta -1**. FIG. 4B shows that in these cells, the growth of which is measured by thymidine incorporation, had been suppressed by **TGF beta -1**. Assay was performed as in FIG. 4A, except that **TGF beta -1** was added at 0.5 ng/ml. This concentration of **TGF beta** induces 50% reduction of [ sup 3 H]-thymidine incorporation in the Mv1Lu cells. The data represent neutralization of **TGF beta** -induced growth inhibition; i.e., [ sup 3 H]-thymidine incorporation in the presence of neither **TGF beta** or decorin=100%; incorporation in the presence of **TGF beta** but not decorin=0%.

#### EXAMPLE IV

##### NEW DECORIN-BINDING FACTOR THAT CONTROLS CELL SPREADING AND...



... of other decorin-associated growth regulatory activities. The overexpressor media were found to contain a **TGF beta** -like growth inhibitory activity. This was shown by gel filtration of the DEAE-isolated decorin... Cy, cytochrome c (Mr=12,400); AP, aprotinin (Mr=6,500); TGF, [ sup 125 I]**TGF beta** -1 (Mr=25,000).

The nature of the growth regulatory activity detected in the low molecular weight fraction was examined with an anti-**TGF beta** -1 antiserum. The antiserum was prepared against a synthetic peptide from residues 78-109 of the human mature **TGF beta** -1. Antisera raised by others against a cyclic form of the same peptide, the terminal...

... residues of which were disulfide-linked, have previously been shown to inhibit the binding of **TGF beta** -1 to its receptors (Flanders et al., Biochemistry 27:739-746 (1988), incorporated by reference binding) of 1:6,000 in radioimmunoassay, bound to **TGF beta** -1 in immunoblots.

This antiserum was capable of inhibiting the activity of purified **TGF beta** -1 on the CHO cells. Moreover, as shown in FIG. 5B, the antiserum also inhibited...

... assay on the CHO cells. Increasing concentrations of an IgG fraction prepared from the anti-**TGF beta** -1 antiserum suppressed the stimulatory effect of the low molecular weight fraction in a concentration ...

...largecircle.)

The above result identified the stimulatory factor in the low molecular weight fraction as **TGF beta** -1. However, **TGF beta** -1 is not the only active compound in that fraction. Despite the restoration of thymidine incorporation by the anti-**TGF beta** -1 antibody shown in FIG. 5B, the cells treated with the low molecular weight fraction were morphologically...recombinant decorin is associated with a cell regulatory factor, MRF, distinct from the well characterized **TGF beta**'s.

Additional evidence that the new factor is distinct from **TGF beta** -1 came from HPLC experiments. Further separations of the low molecular weight from the Sepharose...

... epithelial cells and MRF activity in H-ras 3T3 cells. The result showed that the **TGF beta** -1 activity eluted at the beginning of the gradient, whereas the MRF activity eluted toward...

2/K/61 (Item 61 from file: 654)  
DIALOG(R)File 654:(c) format only 1999 The Dialog Corp. All rts. reserv.

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#### ABSTRACT

... of decorin, a 40,000 dalton protein that usually carries a glycosaminoglycan chain, to bind TGF- beta. The invention also provides a novel cell regulatory factor designated MRF. Also provided are methods...

...sup -3 for molecular weight standards.

FIGS. 2A-12B show binding of [ sup 125 I]-TGF- beta 1 to decorin-Sepharose. FIG. 2A shows fractionation of [ sup 125 I]-TGF- beta 1 by decorin-Sepharose affinity chromatography. [ sup 125 I]-TGF- beta 1 (5X10 sup 5 cpm) was incubated in BSA-coated polypropylene tubes with 0.2...by SDS-polyacrylamide gel under nonreducing conditions. Lane 1: the original [ sup 125 I]-labeled TGF- beta 1 sample; lanes 2-7: flow through and wash fractions; lanes 8-10: 3M NaCl ...

...separating gel.

FIGS. 3A and 3B shows the inhibition of binding of [ sup 125 I]-TGF - beta 1 to decorin by proteoglycans and their core proteins. FIG. 3A shows the competition of [ sup 125 I]-TGF- beta 1 binding to decorin-coated microtiter wells by recombinant decorin (.circle-solid.), decorin isolated from...

... represents the mean of duplicate determinants. FIG. 3B shows the competition of [ sup 125 I]-TGF- beta 1 binding with chondroitinase ...in FIG. 3A.

FIGS. 4A and 4B show neutralization of the growth regulating activity of TGF- beta 1 by decorin. FIG. 4A shows inhibition of TGF- beta 1-induced proliferation of CHO cells by decorin. The [ sup 3 H]Thymidine incorporation assay...

...described in the legend of FIG. 1 in the presence of 5 ng/ml of TGF- beta 1 and the indicated concentrations of purified decorin (.circle-solid.) or BSA ( small circle). At the concentration used, TGF- beta 1 induced a 50% increase of [ sup 3 H]thymidine incorporation in the CHO cells...

... this growth stimulation; i.e. [ sup 3 H]thymidine incorporation in the absence of either TGF- beta 1 or decorin=0%, incorporation in the presence of TGF- beta but not decorin=100%. Each point shows the mean +- standard deviation of triplicate samples. FIG. 4B shows neutralization of TGF- beta 1-induced growth inhibition in MvLu cells by decorin. Assay was performed as in A except that TGF- beta 1 was added at 0.5 ng/ml. This concentration of TGF- beta 1 induces 50% reduction of [ sup 3 H]thymidine incorporation in the MvLu cells. The data represent neutralization of TGF- beta -induced growth inhibition; i.e. [ sup 3 H]thymidine incorporation in the presence of neither TGF- beta or decorin=100%; incorporation in the presence of TGF- beta but not decorin=0%.

FIG. 5A shows separation of growth inhibitory activity from decorin-expressing...

... Cy: cytochrome c (Mr=12,400); Ap: aprotinin (Mr=6,500); TGF: [ sup 125 I]-TGF- **beta** 1 (Mr=25,000).

FIG. 5B shows identification of the growth stimulatory material from gel filtration as TGF- **beta** 1. The growth stimulatory activity from the late fractions ...A) was identified by inhibiting the activity with protein A-purified IgG from an anti-TGF- **beta** antiserum. Data represent percent inhibition of growth stimulatory activity in a [ sup 3 H]thymidine incorporation assay. Each point shows the mean +- standard deviation of triplicate determinations. Anti-TGF- **beta** 1 (.circle-solid.), normal rabbit IgG ( small circle).

FIGS. 6A and 6B show micrographs demonstrating a decorin-binding cell regulatory activity that is not suppressed by antibodies to TGF - **beta** 1.

FIGS. 7A and 7B show that decorin inhibits the binding of [ sup 125 I]-TGF- **beta** to Type III TGF- **beta** receptor ( **beta** glycan) on HepG2 cells. FIG. 7A shows the non-reduced lysate of HepG2...

... reduction of intensity of **beta** glycan band (approximately 300 kDa) and uncross-linked band (free TGF- **beta**, 25 kDa) in the presence of decorin (10,000Xmolar excess) is shown.

FIGS. 8A and 8B show that decorin inhibits the binding of [ sup 125 I]-TGF- **beta** to Type III TGF- **beta** receptor on MG-63 cells. FIG. 8A shows the resolution ...that decorin (DC-9, DC-12) and biglycan inhibit the binding of [ sup 125 I]-TGF- **beta** to immobilized decorin.

FIG. 10 shows the concentration dependence of decorin inhibition of [ sup 125 I]-TGF- **beta** binding to HepG2 cells.

FIG. 11 shows the amino acid sequence of human fibromodulin deduced... shows the binding of radiolabeled proteoglycan fusion proteins and MBP to microtiter wells coated with TGF- **beta** 1. TGF- **beta** 1 was used ...specific activities 2300 difference 2800 Cl/mmol) of the labeled proteins were added to the TGF- **beta** 1-coated wells (total volume 100  $\mu$ l). After incubation for 6 hours at 37 degree(s) C., the wells were washed four times. TGF- **beta** 1-binding was determined by counting the entire wells in a gamma counter and is...

...added to the wells.

FIG. 15 shows the specificity of proteoglycan core protein binding to TGF- **beta** 1. Microtiter wells were coated with the indicated proteins (75  $\mu$ l/well, 3  $\mu$ l...added to the wells.

FIG. 16 shows the time-course of MBP-biglycan binding to TGF- **beta** 1. sup 125 I-MBP-biglycan was added to TGF- **beta** 1 coated wells (75  $\mu$ l, 1  $\mu$ g/ml) at 4 degree(s) C...

... FIG. 17A and 17B show the inhibition of the binding of biglycan fusion protein to TGF- **beta** 1 by proteoglycan fusion proteins and intact proteoglycans. Binding of sup 125 I-MBP-biglycan to TGF- **beta** 1 was measured in the presence of the indicated concentrations of (FIG. 17A) unlabeled MBP...in the absence of competitor.

FIG. 18 shows the competition for the binding of radiolabeled TGF- **beta** 1, - **beta** 2 and - **beta** 3 to microtiter wells coated with biglycan fusion protein. The binding of sup 125 I-labeled TGF- **beta** 1 (solid bars), TGF- **beta** 2 (hatched bars) or TGF- **beta** 3 (open bars) (50,000 cpm/well, specific activities 5,000 to 7,000 Cl...

... nonspecific binding as is expressed as percent ( +- S.D.) of the total amount of labeled TGF- **beta** 1, 2 or 3 that was added to the

wells.

FIGS. 19A and 19B show the competition for the binding of labeled **TGF- beta 1** to MvLu cells by proteoglycan fusion proteins. (FIG. 19A) Subconfluent cultures of MvLu mink lung cells cultured in 48-well plates were incubated with sup 125 I-**TGF- beta 1** (100 pM) in the presence (n.s.) or absence (B sub o) of unlabeled **TGF- beta 1** (20 nM) or the indicated concentrations of proteoglycan fusion proteins in a total volume...

...D., n=3) is expressed as percent of the total amount of sup 125 I-**TGF- beta 1** that was added. (FIG. 19B) Mink lung cells were incubated with sup 125 I-**TGF- beta 1** (100 pM) in the absence or presence of unlabeled **TGF- beta** (20 nM) or MBP-fusion proteins (3 mu M) in 24-well plates. After incubation... by NaDodSO sub 4 -PAGE and autoradiography. Binding in the absence of competitor (a), with **TGF- beta 1** (b), MBP-BG (c), MBP-DEC (d), MBP-FM (e) or MBP (f). The positions of pre-stained marker proteins are indicated. The positions of the **TGF- beta** type ... its signal transduction receptor (Yayon et al., 1991; Rapraeger et al., 1991).

The binding of **TGF- beta** to proteoglycans represents a different type of growth factor-proteoglycan interaction. **TGF- beta** has been demonstrated to bind to the core proteins of at least two proteoglycans. One...

... these proteoglycans is decorin, a small interstitial extracellular matrix proteoglycan that can interact with **TGF- beta** via its core protein (Yamaguchi et al., 1990). Decorin, also known as PG-II or... Biol. 4: 229, (1988); McFarland et al., Science 245: 494 (1989)).

The second type of **TGF- beta** -binding proteoglycan is the type III **TGF- beta** receptor, betaglycan (Segarini and Seyedin et al., 1988; Andres et al., 1989). Betaglycan is a...

... Casillas et al., 1991; Wang et al., 1991) that apparently is not involved in the **TGF- beta** signal transduction pathway but may function as a cell-surface **TGF- beta** reservoir presenting **TGF- beta** to its signal transduction receptors.

Transforming growth factor beta s (**TGF- beta**) are a family of multi-functional cell regulatory factors produced in various forms by many ...

... cells (for review see Sporn et al., J. Cell Biol. 105: 1039, (1987)). Five different **TGF- beta**'s are known, but the functions of only two, **TGF- beta 1** and **TGF- beta 2**, have been characterized in any detail. **TGF- beta**'s are the subject of U.S. Pat. Nos. 4,863,899; 4,816,561; and 4,742,003 which are incorporated by reference. **TGF- beta 1** and **TGF- beta 2** are publicly available through many commercial sources (e.g. R & D Systems, Inc., Minneapolis, Minn.). In some cells, **TGF- beta** promotes cell proliferation, in others it **suppresses** proliferation. A marked effect of **TGF- beta** is that it promotes the production of **extracellular matrix** proteins and their receptors by cells (for review see Keski-Oja et al., J. Cell...

... Roberts and Sporn in "Peptides Growth Factors and Their Receptors" [Springer-Verlag, Heidelberg] (1989)).

While **TGF- beta** has many essential cell regulatory functions, improper **TGF- beta** activity can be detrimental to an organism. Since the growth of mesenchyme and proliferation of mesenchymal cells is stimulated by **TGF- beta**, some tumor cells may use **TGF- beta** as an autocrine growth factor. Therefore, if the growth factor

activity of **TGF- beta** could be prevented, tumor growth could be controlled. In other cases the **inhibition** of cell proliferation by **TGF- beta** may be detrimental, in that it may prevent healing of injured tissues. The stimulation of **extracellular matrix** production by **TGF- beta** is important in situations such as wound healing. However, in some cases the body takes...

...of excessive accumulation of extracellular matrix is glomerulonephritis, a disease with a detrimental involvement of **TGF- beta**.

Thus, there exists a critical need to develop compounds that can modulate the effects of cell regulatory factors such as **TGF- beta**. The present invention satisfies this need and provides related advantages.

#### SUMMARY OF THE INVENTION

The...  
DETAILED DESCRIPTION OF THE INVENTION

Increased **TGF- beta** production has been found to be an important element in a number of fibrotic diseases...

...collagens, and tenascin (Ignatz and Massague, 1986; Varga et al., 1987; Pearson et al., 1988), **TGF- beta** also upregulates the expression of proteoglycans (Bassols and Massague, 1988). In mesangial cells both decorin and biglycan can increase as much as 50-fold after induction by **TGF- beta** (Border et al., 1990a), whereas in fibroblasts only biglycan seems to be elevated (Romaris et al., 1992; Kahari et al., 1991). Fibromodulin has not been studied in this regard. **TGF- beta** plays a pivotal role in the pathogenesis of experimentally induced glomerulonephritis, the most critical manifestation of which is the accumulation of **extracellular matrix** in the glomeruli (Border et al., 1990). A recent study shows that injection of recombinant decorin into glomerulonephritic rats can **suppress** the matrix accumulation (Border et al., 1992). The present invention indicates that fibromodulin can be even more effective in that situation. The **TGF- beta** neutralizing activities of the decorin-type proteoglycans indicates that new types of therapeutics can be...cell surface receptors and include growth factors. Examples of cell regulatory factors include the five **TGF- beta**'s, platelet-derived growth factor, epidermal growth factor, insulin like growth factor I and II...

...be bound by different proteins which can affect the regulatory factor's activity. For example, **TGF- beta** 1 is bound by decorin, biglycan and fibromodulin, and MRF is bound by decorin.

By...be recognized by their ability to competitively inhibit the binding of, for example, decorin to **TGF- beta**, or of other polypeptides containing leucine-rich repeats to their cognate growth factors. As an...

...can be attached to an affinity matrix, as by the method of Example II. Labelled **TGF- beta**, and the fragment in question can then be contacted with the affinity matrix and the amount of **TGF- beta** bound thereto determined.

As used herein, "decorin" refers to a proteoglycan having substantially the structural...a cell regulatory factor. Specifically, the invention teaches the discovery that decorin and biglycan bind **TGF- beta** s and MRF and that such binding can inhibit the cell regulatory functions of **TGF- beta** s. Further, both decorin and biglycan are about 80% homologous and contain a leucine...microtiter assay as set forth in Example II, using known cell regulatory factors, such as **TGF- beta** s. Alternatively, any later discovered cell regulatory factor could be tested, for example by affinity...

...one skilled in the art could simply substitute a novel cell regulatory

factor for a **TGF- beta** or a novel leucine-rich repeat protein for decorin or biglycan in the Examples to...morphology of transformed 3T3 cells, and has an activity which is not inhibited with anti-**TGF-beta 1 antibody**. Additionally, MRF separates from **TGF-beta 1** in HPLC.

The invention still further provides a method of purifying a cell regulatory...

...which becomes bound to the protein. The method can be used, for example, to purify **TGF- beta 1** by using decorin.

The invention additionally provides a method of treating a pathology caused by a **TGF- beta** -regulated activity comprising contacting the **TGF- beta** with a purified polypeptide, wherein the polypeptide comprises the **TGF- beta** binding domain of a protein and wherein the protein is characterized by a leucine-richWhile the method is generally applicable, specific examples of pathologies which can be treated include cancer, a fibrotic disease, and glomerulonephritis. In fibrotic cancer, for example, decorin can be used to bind **TGF- beta**, destroying **TGF- beta's** growth stimulating activity on the cancer cell. Other proliferative pathologies include rheumatoid arthritis, arteriosclerosis...

... of the protein. For example, decorin could be bound by a molecule, such as an **antibody**, which prevents decorin from binding **TGF-beta s**, thus preventing decorin from inhibiting the **TGF-beta s'** activity. Thus, the **TGF- beta s** wound healing activity could be promoted by binding **TGF- beta 1** inhibitors.

In addition, decorin has been found to inhibit the binding of **TGF-beta s** to their receptors. FIGS. 7, 8 and 10 show the results of these studies in which cells bearing **TGF- beta** receptors (betaglycan) were incubated with **TGF- beta** in the presence and absence of decorin.

The present invention further relates to methods for...

... factor believed to induce the deposition of fibrous scar tissue is transforming growth factor- beta (**TGF- beta**). Decorin binds and neutralizes a variety of biological functions of **TGF- beta**, including the induction of extracellular matrix. Due to the lack of elastic property of this...to exhibit essentially no detectable scarring compared to control wounds not treated with decorin. The **TGF- beta** -induced scarring process has been shown to be unique to adults and third trimester human...

... trimesters. The absence of scarring in fetal wounds has been correlated with the absence of **TGF- beta** in the wound bed. In contrast, the wound bed of adult tissue is heavily deposited with **TGF-beta** and the fully healed wound is replaced by a reddened, furrowed scar containing extensively fibrous...which do not substantially affect the activity of the various molecules of this invention including **TGF-beta**, MRF, decorin, biglycan and fibromodulin are also included within the definition of those molecules. It... SDS-polyacrylamide electrophoresis are collected and represent purified decorin core protein.

## EXAMPLE II

### BINDING OF **TGF-.beta.** TO DECORIN

#### a. Affinity Chromatography of **TGF- beta** on Decorin-Sepharose

Decorin and gelatin were coupled to cyanogen bromide-activated Sepharose (Sigma) by... Biol. Chem. 259: 10995-11000 (1984)) which is incorporated herein by reference and the labeled **TGF- beta** was separated from the unreacted iodine by gel filtration on Sephadex G-25, equilibrated

with...

... buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) (FIG. 2). [ sup 125 I]-TGF- beta 1 (5X10 sup 5 cpm) was incubated in BSA-coated polypropylene tubes with 0.2 shown in FIG. 2B. The TGF- beta 1 starting material contains a major band at 25 kd. This band represents the native TGF- beta 1 dimer. In addition, there are numerous minor bands in the preparation. About 20-30...

... Sepharose nonbound fraction contains all of the minor components and some of the 25 kd TGF- beta 1, whereas the bound, urea-eluted fraction contains only TGF- beta 1 (FIG. 2B). These results show that TGF- beta 1 binds specifically to decorin, since among the various components present in the original TGF- beta 1 preparation, only TGF- beta 1 bound to the decorin-Sepharose affinity matrix and since there was very little binding to the control gelatin-Sepharose affinity matrix. The TGF- beta 1 that did not bind to the decorin-Sepharose column may have been denatured by the iodination. Evidence for this possibility was provided by affinity chromatography of unlabeled TGF- beta 1 as described below.

In a second experiment, unlabeled TGF- beta 1 180 ng was fractionated on decorin-Sepharose as described above for sup 125 I-TGF- beta.

TGF- beta 1 (180 ng) was incubated with decorin-Sepharose or BSA-agarose (0.2 ml packed...

... inhibition of [ sup 3 H]thymidine incorporation in MvLu cells (Example III). The amounts of TGF- beta 1 in each pool were calculated from the standard curve of [ sup 3 H]thymidine incorporation obtained from a parallel experiment with known concentration of TGF- beta 1. The results show that the TGF- beta 1 bound essentially quantitatively to the decorin column, whereas there was little binding to the control column (Table 1). The partial recovery of the TGF- beta 1 activity may be due to loss of TGF- beta 1 in the dialyses.

TABLE I Decorin-Sepharose affinity chromatography of nonlabeled TGF- beta 1 monitored by growth inhibition assay in MvLu cells.

Elution	TGF- beta 1 (ng)	Decorin-Sepharose	BSA-Sepharoseb.	Binding of TGF- beta 1 to Decorin in a Microtiter Assay: Inhibition by Core Protein and Biglycan

The binding of TGF- beta 1 to decorin was also examined in a microtiter binding assay. To perform the assay...05% Tween (PBS/Tween) and samples containing 5X10 sup 4 cpm of [ sup 125 I]-TGF- beta 1 and various concentrations of competitors in PBS/Tween were added to each well. The...

... under the conditions used. Nonspecific binding, determined by adding 100-fold molar excess of unlabeled TGF- beta 1 over the labeled TGF- beta 1 to the incubation mixture, was about 13% of total binding. This assay was also...decorin and decorin isolated from bovine skin (PGII) inhibited the binding of [ sup 125 I]-TGF- beta 1, as expected (FIG. 3A). Biglycan isolated from bovine articular cartilage was as effective an... chondroitinase ABC did not shown any inhibition. Additional binding experiments showed that [ sup 125 I]-TGF- beta 1 bound to microtiter wells coated with biglycan or its chondroitinase-treated core protein. These results show that TGF- beta 1 binds to the core protein of decorin and biglycan and implicates the leucine-rich...

...EXAMPLE III

# ANALYSIS OF THE EFFECT OF DECORIN ON CELL PROLIFERATION STIMULATED OR INHIBITED BY TGF- $\beta$ 1

The ability of decorin to modulate the activity of TGF- $\beta$ 1 was examined in [<sup>3</sup>H]thymidine incorporation assays. In one assay, an unamplified... H]thymidine incorporation was assayed as described (Cheifetz et al., Cell 48: 409-415 (1987)). TGF- $\beta$ 1 was added to the CHO cell cultures at 5 ng/ml. At this concentration...

... concentrations. The results are shown in FIG. 4A. The data represent percent neutralization of the TGF- $\beta$ 1-induced growth stimulation, i.e., [<sup>3</sup>H]thymidine incorporation, in the absence of either TGF- $\beta$ 1 or decorin=0%, incorporation in the presence of TGF- $\beta$ 1 but not decorin=100%. Each point shows the mean  $\pm$  standard deviation of triplicate samples. Decorin (circle-solid.) BSA (small circle).

Decorin neutralized the growth stimulatory activity of TGF- $\beta$ 1 with a half maximal activity at about 5  $\mu$ g/ml. Moreover, additional decorin suppressed the [<sup>3</sup>H]-thymidine incorporation below the level observed without any added TGF- $\beta$ 1, demonstrating that decorin also inhibited TGF- $\beta$ 1 made by the ...CHO cells themselves. Both the decorin-expressor and control CHO cells produced an apparently active TGF- $\beta$ 1 concentration of about 0.25 ng/ml concentration into their conditioned media as determined by...

... interference from the decorin in the culture media because, as shown below, the effect of TGF- $\beta$ 1 on the mink cells was not substantially inhibited at the decorin concentrations present in the...

... Type Culture Collection CCL 64) also revealed an effect by decorin on the activity of TGF- $\beta$ 1. FIG. 4B shows that in these cells, the growth of which is measured by thymidine incorporation, had been suppressed by TGF- $\beta$ 1. Assay was performed as in FIG. 4A, except that TGF- $\beta$ 1 was added at 0.5 ng/ml. This concentration of TGF- $\beta$ 1 induces 50% reduction of [<sup>3</sup>H]-thymidine incorporation in the MvLu cells. The data represent neutralization of TGF- $\beta$ 1-induced growth inhibition; i.e., [<sup>3</sup>H]-thymidine incorporation in the presence of neither TGF- $\beta$ 1 or decorin=100%; incorporation in the presence of TGF- $\beta$ 1 but not decorin=0%.

## EXAMPLE IV

### NEW DECORIN-BINDING FACTOR THAT CONTROLS CELL SPREADING AND...

... of other decorin-associated growth regulatory activities. The overexpressor media were found to contain a TGF- $\beta$ 1-like growth inhibitory activity. This was shown by gel filtration of the DEAE-isolated decorin... Cy, cytochrome c (Mr=12,400); AP, aprotinin (Mr=6,500); TGF, [<sup>125</sup>I]-TGF- $\beta$ 1 (Mr=25,000).

The nature of the growth regulatory activity detected in the low molecular weight fraction was examined with an anti-TGF- $\beta$ 1 antiserum. The antiserum was prepared against a synthetic peptide from residues 78-109 of the human mature TGF- $\beta$ 1. Antisera raised by others against a cyclic form of the same peptide, the terminal... residues of which were disulfide-linked, have previously been shown to inhibit the binding of TGF- $\beta$ 1 to its receptors (...this work has a titer (50% binding) of 1: 6,000 in radioimmunoassay, bound to TGF- $\beta$ 1 in immunoblots.

This antiserum was capable of inhibiting the activity of purified TGF- $\beta$ 1 on the CHO cells. Moreover, as shown in FIG. 5A and 5B, the antiserum...



... assay on the CHO cells. Increasing concentrations of an IgG fraction prepared from the anti-TGF- **beta** 1 antiserum suppressed the stimulatory effect of the low molecular weight fraction in a concentration ...circle).

The above result identified the stimulatory factor in the low molecular weight fraction as TGF- **beta** 1. However, TGF- **beta** 1 is not the only active compound in that fraction. Despite the restoration of thymidine incorporation by the anti-TGF- **beta** 1 antibody shown in FIG. 5A and 5B, the cells treated with the low molecular weight fraction...

... recombinant decorin is associated with a cell regulatory factor, MRF, distinct from the well characterized TGF- **beta**'s.

Additional evidence that the new factor is distinct from TGF- **beta** 1 came from HPLC experiments. Further separations of the low ... epithelial cells and MRF activity in H-ras 3T3 cells. The result showed that the TGF- **beta** 1 activity eluted at the beginning of the gradient, whereas the MRF activity eluted toward the end of the gradient.

#### EXAMPLE V

##### INHIBITION OF TGF-**beta**. BINDING

###### A. Cross Linking of [ sup 125 I]-TGF- **beta** to HepG2 Cells

About 2.5X10 sup 4 HepG2 cells (human hepatocellular carcinoma, ATCC No. HB 8065) were incubated with 100 pM [ sup 125 I]-TGF- **beta** in the presence of recombinant decorin, TGF- **beta**, or alpha - TGF- **beta** antibody for 2 hours at room temperature. Cells were washed four times prior to suspension in...were resolved on 4-12% SDS-PAGE under reducing and non-reducing conditions. Cross-linked TGF - **beta** was visualized by autoradiography.

FIGS. 7A and 7B show the results of the studies. Decorin inhibits the binding of TGF- **beta** to beta glycan, a TGF- **beta** receptor found on HepG2 cells.

###### B. Cross Linking of [ sup 125 I]-TGF- **beta** to MG-63 Cells

About 10 sup 5 MG-63 cells (male osteosarcoma, ATCC No. CRL 1427) were incubated with 150 pM [ sup 25 I]-TGF- **beta** in the presence of a recombinant decorin preparation (designated as DC-13) or TGF- **beta** for 2 hours at room temperature. Cells were washed four times in ice cold binding... results of the studies. Similar to the above studies with HepG2 cells, decorin also inhibits TGF- **beta** binding to its receptors on the MG-63 cells.

###### C. Binding Studies of sup 125 I-TGF- **beta** to Immobilized Decorin

A 96-well Linbro microtiter plate was coated with 0.5 mu...

...times with 200 mu l PBS (0.15M NaCl) per well to remove unbound decorin. TGF- **beta** labeled with sup 125 I (400 pM, New England Nuclear, Bolton-Hunter Labeled) was pre...control; and biglycan is recombinant human biglycan.

Fifty mu l/well of the pre-incubated TGF- **beta** mixture or control were added and incubated overnight at 0 degree(s) C. Following the incubation, 50 mu l of the free TGF- **beta** supernatants were transferred to labeled tubes. The plate was washed 3 times with 0.05...

...30 minutes. While gently pulsing the solution, 100 mu l of bound sup 125 I-TGF- **beta** was removed from each well and transferred into

tubes for counting in a gamma counter. The 50  $\mu$ l free **TGF- $\beta$**  samples were counted in parallel to the 100  $\mu$ l bound **TGF- $\beta$**  samples to obtain the bound: free ratio. The results of the binding studies with immobilized decorin are summarized in FIG. 9.

#### D. Binding of sup 125 I-TGF- $\beta$ to HepG2 Cells

About  $2.5 \times 10^4$  HepG2 cells were incubated with 250 pM [ sup 125 I]-**TGF- $\beta$** , in the presence of recombinant human decorin (DC-12) or PT-71 (MBP) for 2...

... summarized in FIG. 10. Table II provides numerical data for decorin (DC-12) inhibition of **TGF- $\beta$**  binding to HepG2 cells. The "% Change" represents the difference in the mean cpm of the test samples compared to the cpm of the medium (negative control). The alpha -**TGF- $\beta$**  antibody inhibits the binding of labeled **TGF- $\beta$**  to cells bearing **TGF- $\beta$**  receptors and serves as a positive control.

TABLE II BINDING OF 125I-TGF- $\beta$  1 TO HEPG2

CELLS			
CPM			%
Treatment	Concentration	Bound	Mean
		2,800	2,812 $\pm$ 275
			-78
Anti-	2.5 X 10E-7M	6,191	
<b>TGF-<math>\beta</math></b> 1		4,848	
(R&D)		3,839	4,959 $\pm$ 1,180
			-61

Decorin 2.5... HepG2 cells obtained from subconfluent cultures were incubated

with 250 pM 125I**TGF- $\beta$**  1 and **TGF  $\beta$** , anti **TGF  $\beta$** , decorin, or decorin fragments for 2 hours at room temperature.

\*\*Unbound 125I**TGF- $\beta$**  1 was...human lung fibroblasts (ATCC Accession No. CCL 75; Rockville, Md.) that had been exposed to **TGF- $\beta$**  1 (3 ng/ml) for 12 hours. Total cellular RNA (1  $\mu$ g) was reverse...specific activities ranged from 2300 to 2800 Ci/mmol, with radiochemical purities greater than 95%. **TGF- $\beta$**  1, 2 AND 3 (1-5  $\mu$ g) were labeled as described above using O...

... performed incubating radiolabeled MBP-proteoglycan fusion proteins in microtiter wells coated with increasing amounts of **TGF- $\beta$**  1.

Immunon-2 microtiter wells (Dynatech; Chantilly, Va.) were coated with **TGF- $\beta$**  1 (75  $\mu$ l, 1  $\mu$ g/ml) or other proteins dissolved in 0.1M... the wells was less than 5% of the total radioactivity added. The coating efficiency for **TGF- $\beta$**  1 was 58.7  $\pm$  0.5% (n=3), giving approximately 44 ng of **TGF- $\beta$**  1 per well. The coating efficiency was calculated by adding a small amount of sup 125 I-labeled **TGF- $\beta$**  1 to the coating solution and determining the surface-associated radioactivity after the overnight incubation MBP-BG), MBP-decorin (MBP-DEC) and MBP-fibromodulin (MBP-FM) bound to **TGF- $\beta$**  1-coated wells in a concentration-dependent manner, displaying maximum binding of 50%, 20%, and 55%, respectively (FIG. 14). Radiolabeled MBP alone did not bind to the **TGF- $\beta$**  1-coated wells.

The binding of the radiolabeled fusion proteins was specific for **TGF- $\beta$** , since little to no binding was observed to immobilized NGF, EGF, insulin or platelet factor 4. MBP-FM bound slightly to immobilized **TGF- $\beta$**  1 precursor protein, but MBP-BG and MBP-DEC did not (FIG. 15).

Since the biglycan fusion protein, MBP-biglycan, showed high binding activity toward **TGF- beta 1**, it was used to characterize further the proteoglycan-**TGF- beta 1** interactions. The binding of MBP-BG to **TGF- beta 1** was time- and temperature-dependent (FIG. 16). Binding increased rapidly at 37 degree(s)...

...Unlabeled MBP-BG competed for the binding of sup 125 I-labeled MBP-BG to **TGF- beta 1** in a concentration-dependent manner (FIG. 17A). MBP- DEC and MBP-FM competed for the binding of labeled MBP-BG to **TGF- beta 1**. They were about equally effective competitors as MBP-BG, yielding half-maximal inhibitory concentrations...

... binding site concentrations ( $B_{sub max}$ ) for the interaction of the proteoglycan fusion proteins with **TGF- beta 1**. Best results were obtained for two-site binding models with  $K_{sub d}$  values...

...  $6 \times 10^{-12}$  moles per well for the low affinity binding sites, respectively. Given a **TGF- beta 1** coating concentration of 1  $\mu$ g/ ...efficiency of about 60%, these values indicate a molar ratio between proteoglycan fusion protein and **TGF- beta 1** of one to ten for the high affinity binding site and one to one...

... the low affinity binding site, respectively. Proteoglycans were also tested for their ability to bind **TGF- beta 2** and **TGF- beta 3**, the other known mammalian isoforms of **TGF- beta**. Binding of **TGF- beta 1**, 2, and 3 to immobilized MBP-biglycan was inhibited by all three fusion proteins and all three intact proteoglycans (FIG. 18). **TGF- beta 3** binding to MBP-BG was more effectively inhibited by decorin and biglycan than fibromodulin...

... sequences of human biglycan, decorin and fibromodulin compete for binding of labeled MBP-biglycan to **TGF- beta 1** with similar affinities, indicating that functionally highly conserved regions of the core proteins are involved in the binding to **TGF- beta**. The fact that bacterially produced recombinant proteoglycan core proteins had similar activities to recombinant decorin presence of the **TGF- beta** binding activity in the core proteins of these proteoglycans.

#### EXAMPLE XI

##### CELL BINDING EXPERIMENTS

The ability of the proteoglycan fusion proteins to compete for **TGF- beta 1** binding to cells was tested in cell-binding experiments. Cell binding experiments were performed...

... in DMEM containing 10% FCS were used in binding experiments. Cells were incubated with labeled **TGF- beta 1** in the presence or absence of unlabeled **TGF- beta 1** or proteoglycan fusion proteins. The cells were washed twice with ice-cold binding buffer...

... binding buffer for 30 minutes at 4 degree(s) C. to remove endogenous receptor-associated **TGF- beta**. Samples containing labeled and unlabeled proteins were added to the wells in a total volume...determined by counting a portion of the solubilized cells in a gamma counter.

Whereas unlabeled **TGF- beta 1** competed effectively for the binding of labeled **TGF- beta 1** to all three types of **TGF- beta** receptors, much higher concentrations of the proteoglycan fusion proteins were needed to compete for **TGF- beta 1** binding to these cells (FIG. 19A). Half-maximal competition was achieved by fusion protein concentrations averaging about 3  $\mu$ M.

#### EXAMPLE XII

##### CROSS-LINKING OF **TGF- beta**. TO RECEPTORS

For receptor cross-linking, cells were grown in 24-well culture dishes and...ray film for several days at -70 degree(s) C.

Cross-linking experiments revealed that **TGF- beta 1** binding to the type I and type III receptors was affected more by all...

... FIG. 19B). Laser densitometry analyses of the respective autoradiograms showed that the binding of labeled **TGF- beta 1** to type I and III receptors was decreased by approximately 25% or 50%, respectively, at the proteoglycan concentration used.

Competition was most effective for **TGF- beta** binding to the type I and type III **TGF- beta** receptors, perhaps because these receptors have a lower affinity for **TGF- beta** than does the type II receptor. While the affinities of the proteoglycans for the **TGF- beta** are much lower than those of any of the receptors, all of the proteoglycans are...

... the experimental conditions used in the cell binding experiments do not favor the binding of **TGF- beta** to proteoglycans.

Although the invention has been described with reference to the presently-preferred embodiments...

2/K/70 (Item 70 from file: 654)  
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#### ABSTRACT

The present invention provides a method of inhibiting an activity of **TGF beta** comprising contacting the **TGF beta** with a purified decorin. In a specific embodiment, the present invention relates to the ability of decorin, a 40,000 dalton protein that usually carries a glycosaminoglycan chain, to bind **TGF beta**. The invention also provides a novel cell regulatory factor designated MRF. Also provided are methods...

...3 for molecular weight standards.

FIGS. 2A and 2B show binding of [ sup 125 I]**TGF beta 1** to decorin-Sepharose.

(FIG. 2A) Fractionation of [ sup 125 I]-**TGF beta 1** by decorin-Sepharose affinity chromatography. [ sup 125 I]**TGF beta 1** (5X10 sup 5 cpm) was incubated in BSA-coated polypropylene tubes with 0.2 ...

... by SDS-polyacrylamide gel under nonreducing conditions. Lane 1: the original [ sup 125 I]-labeled **TGF beta 1** sample; lanes 2-7: flow through and wash fractions; lanes 8-10: 3M NaCl...

...separating gel.

FIGS. 3A and 3B show the inhibition of binding of [ sup 125 I]**TGF beta 1** to decorin by proteoglycans and their core proteins.

(FIG. 3A) Competition of [ sup 125 I]**TGF beta 1** binding to decorin-coated microtiter wells by recombinant decorin (.circle-solid.), decorin isolated from...  
...Each point represents the mean of duplicate determinants.

(FIG. 3B) Competition of [ sup 125 I]**TGF beta 1** binding with chondroitinase ABC-treated proteoglycans and BSA. The concentrations of competitors were expressed...

...in FIG. 3A.

FIGS. 4A and 4B show neutralization of the growth regulating activity of **TGF beta 1** by decorin.

(FIG. 4A) Shows inhibition of **TGF beta 1**-induced proliferation of CHO cells by decorin. [ sup 3 H]Thymidine incorporation assay was...

...described in the legend of FIG. 1 in the presence of 5 ng/ml of **TGF beta -1** and the indicated concentrations of purified decorin (.circle-solid.) or BSA (0). At the concentration used, **TGF beta -1** induced a 50% increase of [ sup 3 H]thymidine incorporation in the CHO cells...

... this growth stimulation; i.e. [ sup 3 H]thymidine incorporation in the absence of either **TGF beta 1** or decorin=0%, incorporation in the presence of **TGF beta** but not decorin=100%. Each point shows the mean +- standard deviation of triplicate samples.

(FIG. 4B) Shows neutralization of **TGF beta 1**-induced growth inhibition in Mv1Lu cells by decorin. Assay was performed as in A except that **TGF beta -1** was added at 0.5 ng/ml. This concentration of **TGF beta -1** induces 50% reduction of [ sup 3 H]thymidine incorporation in the Mv1Lu cells. The data represent neutralization of **TGF beta**-induced growth inhibition; i.e. [ sup 3 H]thymidine incorporation in the presence of neither **TGF beta** or decorin=100%; incorporation in the presence of **TGF beta** but not decorin=0%.

FIG. 5A shows separation of growth inhibitory activity from decorin-expressing... Cy: cytochrome c (Mr=12,400); Ap: aprotinin (Mr=6,500); **TGF**: [ sup 125 I]**TGF beta 1** (Mr=25,000).

FIG. 5B shows identification of the growth stimulatory material from gel filtration as **TGF beta 1**. The growth stimulatory activity from the late fractions from Sepharose 6B (bar in FIG. 5A) was identified by inhibiting the activity with protein A-purified IgG from an anti-**TGF beta** antiserum. Data represent percent inhibition of growth stimulatory activity in a [ sup 3 H]thymidine incorporation assay. Each point shows the mean +- standard deviation of triplicate determinations. Anti-**TGF beta 1** (.circle-solid.), normal rabbit IgG (.largecircle.).

FIGS. 6A and 6B show micrographs demonstrating a decorin-binding cell regulatory activity that is not suppressed by antibodies to **TGF beta -1**.

...4:229, (1988); McFarland et al., Science 245:494 (1989)).

Transforming growth factor beta's (TGF beta) are a family of multifunctional cell regulatory factors produced in various forms by many types...

... cells (for review see Sporn et al., J. Cell Biol. 105:1039, (1987)). Five different TGF beta's are known, but the functions of only two, TGF beta -1 and TGF beta -2, have been characterized in any detail. TGF beta's are the subject of U.S. Pat. Nos. 4,863,899; 4,816,561; and 4,742,003 which are incorporated by reference. TGF beta -1 and TGF beta -2 are publicly available through many commercial sources (e.g. R & D Systems, Inc., Minneapolis, Minn.). These two proteins have similar functions and will be here collectively referred to as TGF beta. TGF beta binds to cell surface receptors possessed by essentially all types of cells, causing profound changes in them. In some cells, TGF beta promotes cell proliferation, in others it suppresses proliferation. A marked effect of TGF beta is that it promotes the production of extracellular matrix proteins and their receptors by cells (for review see Keski-Oja et al., J. Cell...

...Sporn in "Peptides Growth Factors and Their Receptors" [Springer-Verlag, Heidelberg] in press (1989)).

While TGF beta has many essential cell regulatory functions, improper TGF beta activity can be detrimental to an organism. Since the growth of mesenchyme and proliferation of mesenchymal cells is stimulated by TGF beta, some tumor cells may use TGF beta as an autocrine growth factor. Therefore, if the growth factor activity of TGF beta could be prevented, tumor growth could be controlled. In other cases the inhibition of cell proliferation by TGF beta may be detrimental, in that it may prevent healing of injured tissues. The stimulation of extracellular matrix production by TGF beta is important in situations such as wound healing. However, in some cases the body takes...

...of excessive accumulation of extracellular matrix is glomerulonephritis, a disease with a detrimental involvement of TGF beta.

Thus, there exists a critical need to develop compounds that can modulate the effects of cell regulatory factors such as TGF beta. The present invention satisfies this need and provides related advantages.

#### SUMMARY OF THE INVENTION

The... of decorin, a 40,000 dalton protein that usually carries a glycosaminoglycan chain, to bind TGF beta. The invention also provides a novel cell regulatory factor designated Morphology Restoring Factor, (MRF). Also...

... cell surface receptors and include growth factors. Examples of cell regulatory factors include the five TGF beta 's, platelet-derived growth factor, epidermal growth factor, insulin like growth factor I and II...

... be bound by different proteins which can affect the regulatory factor's activity. For example, TGF beta -1 is bound by decorin and biglycan, and MRF by decorin.

By "cell regulatory factor..."

... be recognized by their ability to competitively inhibit the binding of, for example, decorin to TGF beta, or of other polypeptides containing leucine-rich repeats to their cognate growth factors. As an...

... can be attached to an affinity matrix, as by the method of Example II. Labelled TGF beta, and the fragment in question can then be

contacted with the affinity matrix and the amount of **TGF beta** bound thereto determined.

As used herein, "decorin" refers to a proteoglycan having substantially the structural...

... a cell regulatory factor. Specifically, the invention teaches the discovery that decorin and biglycan bind **TGF beta -1** and MRF and that such binding can inhibit the cell regulatory functions of **TGF beta -1**. Further, both decorin and biglycan are about 80% homologous and contain a leucine-rich...microtiter assay as set forth in Example II, using known cell regulatory factors, such as **TGF beta -1**. Alternatively, any later discovered cell regulatory factor could be tested, for example by affinity...

... Moreover, one skilled in the art could simply substitute a novel cell regulatory factor for **TGF beta -1** or a novel leucine-rich repeat protein for decorin or biglycan in the Examples...

... morphology of transformed 3T3 cells, and has an activity which is not inhibited with anti-**TGF beta -1 antibody**. Additionally, MRF separates from **TGF beta -1** in HPLC.

The invention still further provides a method of purifying a cell regulatory...

...which becomes bound to the protein. The method can be used, for example, to purify **TGF beta -1** by using decorin.

The invention additionally provides a method of treating a pathology caused by a **TGF beta** -regulated activity comprising contacting the **TGF beta** with a purified polypeptide, wherein the polypeptide comprises the **TGF beta** binding domain of a protein and wherein the protein is characterized by a leucine-rich...

... or reduced. While the method is generally applicable, specific examples of pathologies which can be treated include a cancer, a fibrotic disease, and glomerulonephritis. In cancer, for example, decorin can be used to bind **TGF beta -1**, destroying **TGF beta -1**'s growth stimulating activity on the cancer cell.

Finally, a method of preventing the...

... of the protein. For example, decorin could be bound by a molecule, such as an antibody, which prevents decorin from binding **TGF beta -1**, thus preventing decorin from inhibiting the **TGF beta -1** activity. Thus, the **TGF beta -1** wound healing activity could be promoted by binding **TGF beta -1** inhibitors.

It is understood that modifications which do not substantially affect the activity of the various molecules of this invention including **TGF beta** , MRF, decorin, biglycan and fibromodulin are also included within the definition of those molecules. It... SDS-polyacrylamide electrophoresis are collected and represent purified decorin core protein.

#### EXAMPLE II

##### Binding of **TGF.beta.** to Decorin

###### a. Affinity Chromatography of **TGF beta** on Decorin-Sepharose

Decorin and gelatin were coupled to cyanogen bromide-activated Sepharose (Sigma) by...

... of protein per ml of Sepharose matrix according to the manufacturer's instructions. Commercially obtained **TGF beta -1** (Calbiochem, La Jolla, Calif.) was sup 125 I-labelled by the chloramine T method...

... Biol. Chem. 259:10995-11000 (1984)) which is incorporated herein by reference and the labeled **TGF beta** was separated from the unreacted iodine by gel filtration on Sephadex G-25, equilibrated with...  
 ... buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) (FIG. 2A). [ sup 125 I]-**TGF beta** 1 (5X10 sup 5 cpm) was incubated in BSA-coated polypropylene tubes with 0.2...columns and the SDS-PAGE analysis of the fractions is shown in FIG. 2B. The **TGF beta** -1 starting material contains a major band at 25 kd. This band represents the native **TGF beta** -1 dimer. In addition, there are numerous minor bands in the preparation. About 20-30...

... Sepharose nonbound fraction contains all of the minor components and some of the 25 kd **TGF beta** -1, whereas the bound, urea-eluted fraction contains only **TGF beta** -1 (FIG. 2B). These results show that **TGF beta** -1 binds specifically to decorin, since among the various components present in the original **TGF beta** -1 preparation, only **TGF beta** -1 bound to the decorin-Sepharose affinity matrix and since there was very little binding to the control gelatin-Sepharose affinity matrix. The **TGF beta** -1 that did not bind to the decorin-Sepharose column may have been denatured by the iodination. Evidence for this possibility was provided by affinity chromatography of unlabeled **TGF beta** -1 as described below.

In a second experiment, unlabeled **TGF beta** -1 180 ng was fractionated on decorin-Sepharose as described above for sup 125 I-**TGF beta**.

**TGF beta** -1 (180 ng) was incubated with decorin-Sepharose or BSA-agarose (0.2 ml packed...

... inhibition of [ sup 3 H]thymidine incorporation in Mv1Lu cells (Example III). The amounts of **TGF beta** -1 in each pool were calculated from the standard curve of [ sup 3 H]thymidine incorporation obtained from a parallel experiment with known concentration of **TGF beta** -1. The results show that the **TGF beta** -1 bound essentially quantitatively to the decorin column, whereas there was little binding to the control column (Table 1). The partial recovery of the **TGF beta** -1 activity may be due to loss of **TGF beta** -1 in the dialyses.

TABLE I Decorin-Sepharose affinity chromatography of nonlabeled **TGF beta** -1 monitored by growth inhibition assay in Mv1Lu cells.

	<b>TGF beta</b> -1 (ng)
Elution	Decorin-Sepharose
	BSA-Sepharoseb. Binding of <b>TGF beta</b>
-1 to Decorin in a Microtiter Assay: Inhibition by Core Protein and	
Biglycan	

The binding of **TGF beta** -1 to decorin was also examined in a microtiter binding assay. To perform the assay...05% Tween (PBS/Tween) and samples containing 5X10 sup 4 cpm of [ sup 125 I]-**TGF beta** -1 and various concentrations of competitors in PBS/Tween were added to each well. The...

... under the conditions used. Nonspecific binding, determined by adding 100-fold molar excess of unlabeled **TGF beta** -1 over the labeled **TGF beta** -1 to the incubation mixture, was about 13% of total binding. This assay was also...

... decorin and decorin isolated from bovine skin (PGII) inhibited the binding of [ sup 125 I]-**TGF beta** -1, as expected (FIG. 3A). Biglycan isolated from bovine articular cartilage was as effective an...



... chondroitinase ABC did not shown any inhibition. Additional binding experiments showed that [ sup 125 I]-TGF beta -1 bound to microtiter wells coated with biglycan or its chondroitinase-treated core protein. These results show that TGF beta -1 binds to the core protein of decorin and biglycan and implicates the leucine-rich...

#### ...EXAMPLE III

Analysis of the Effect of Decorin on Cell Proliferation Stimulated or Inhibited by TGF.beta.-1

The ability of decorin to modulate the activity of TGF beta -1 was examined in [ sup 3 H]thymidine incorporation assays. In one assay, an unamplified...

... H]thymidine incorporation was assayed as described (Cheifetz et al., Cell 48:409-415 (1987)). TGF beta -1 was added to the CHO cell cultures at 5 ng/ml. At this concentration...

... concentrations. The results are shown in FIG. 4A. The data represent percent neutralization of the TGF beta -1-induced growth stimulation, i.e., [ sup 3 H]thymidine incorporation, in the absence of either TGF beta -1 or decorin=0%, incorporation in the presence of TGF beta -1 but not ...of triplicate samples. Decorin (.circle-solid.) BSA (0).

Decorin neutralized the growth stimulatory activity of TGF beta -1 with a half maximal activity at about 5 mu g/ml. Moreover, additional decorin suppressed the [ sup 3 H]-thymidine incorporation below the level observed without any added TGF beta -1, demonstrating that decorin also inhibited TGF beta made by the CHO cells themselves. Both the decorin-expressor and control CHO cells produced an apparently active TGF beta concentration of about 0.25 ng/ml concentration into their conditioned media as determined by...

... interference from the decorin in the culture media because, as shown below, the effect of TGF beta on the mink cells was not substantially inhibited at the decorin concentrations present in the...

... American Type Culture Collection CCL64) also revealed an effect by decorin on the activity of TGF beta -1. FIG. 4B shows that in these cells, the growth of which is measured by thymidine incorporation, had been suppressed by TGF beta -1. Assay was performed as in FIG. 4A, except that TGF beta -1 was added at 0.5 ng/ml. This concentration of TGF beta induces 50% reduction of [ sup 3 H]-thymidine incorporation in the Mv1Lu cells. The data represent neutralization of TGF beta -induced growth inhibition; i.e., [ sup 3 H]-thymidine incorporation in the presence of neither TGF beta or decorin=100%; incorporation in the presence of TGF beta but not decorin=0%.

#### EXAMPLE IV

New Decorin-Binding Factor that Controls Cell Spreading and...

... of other decorin-associated growth regulatory activities. The overexpressor media were found to contain a TGF beta -like growth inhibitory activity. This was shown by gel filtration of the DEAE-isolated decorin...

... Cy, cytochrome c (Mr=12,400); AP, aprotinin (Mr=6,500); TGF, [ sup 125 I]TGF beta -1 (Mr=25,000).

The nature of the growth regulatory activity detected in the low molecular weight fraction was examined with an anti-TGF beta -1 antiserum. The antiserum was prepared against a synthetic peptide from residues 78-109 of the human mature TGF beta -1. Antisera

raised by others against a cyclic form of the same peptide, the terminal...

... residues of which were disulfide-linked, have previously been shown to inhibit the binding of **TGF beta -1** to its receptors (Flanders et al., Biochemistry 27:739-746 (1988), incorporated by reference...this work has a titer (50% binding) of 1:6,000 in radioimmunoassay, bound to **TGF beta -1** in immunoblots.

This antiserum was capable of inhibiting the activity of purified **TGF beta -1** on the CHO cells. Moreover, as shown in FIG. 5B, the antiserum also inhibited...

... assay on the CHO cells. Increasing concentrations of an IgG fraction prepared from the anti-**TGF beta -1** antiserum suppressed the stimulatory effect of the low molecular weight fraction in a concentration ...

...largecircle.).

The above result identified the stimulatory factor in the low molecular weight fraction as **TGF beta -1**. However, **TGF beta -1** is not the only active compound in that fraction. Despite the restoration of thymidine incorporation by the anti-**TGF beta -1** antibody shown in FIG. 5B, the cells treated with the low molecular weight fraction were morphologically...  
... recombinant decorin is associated with a cell regulatory factor, MRF, distinct from the well characterized **TGF beta**'s.

Additional evidence that the new factor is distinct from **TGF beta -1** came from HPLC experiments. Further separations of the low molecular weight from the Sepharose...

... epithelial cells and MRF activity in H-ras 3T3 cells. The result showed that the **TGF beta -1** activity eluted at the beginning of the gradient, whereas the MRF activity eluted toward...

What is claimed is:

1. A method of inhibiting an activity of **TGF beta**, comprising contacting **TGF beta** with an effective amount of purified decorin.

2. The method of claim 1, wherein the **TGF beta** is **TGF beta -1**.

3. The method of claim 1, wherein the **TGF beta** is **TGF beta -2**.

4. The method of claim 1, wherein the inhibited activity is promotion of cell proliferation.

5. The method of claim 1, wherein the inhibited activity is suppression of cell proliferation.

6. The method of claim 1, wherein the activity is promotion of extracellular matrix production.

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... cause a perfusion of, e.g., insulin in the blood of a person suffering from diabetes. However, for many therapeutic agents there is no satisfactory method of either site-specific or systemic administration.

In addition...  
... or cerebrovascular diseases where angiogenic factors may improve

circulation to the brain or other tissues. **Diabetes** mellitus may be **treated** by introduction of glucose-responsive insulin secreting cells in the portal circulation where the liver...for recollateralization agents, such as transforming growth factor alpha (TGF- alpha), transforming growth factor beta (TGF- **beta** ) angiogenin, tumor necrosis factor alpha, tumor necrosis factor beta, acidic fibroblast growth factor or basic fibroblast growth factor can be used. In the **treatment** of vasomotor diseases, genetic material coding for vasodilators or vasoconstrictors may be used. These include atrial natriuretic factor, platelet-derived growth factor or endothelin. In the **treatment** of **diabetes**, genetic material coding for insulin may be used.

The present invention can also be used in the **treatment** of malignancies by placing the transformed cells in proximity to the malignancy. In this application... 4, 3, 6 or 8, inhibitors/inducers of differentiation, such as TNF- alpha or beta, **TGF- beta** (1, 2 or 3), IL-1, soluble growth factor receptors (PDGF, FGF receptors), recombinant **antibodies** to growth factors or receptors, analogs of growth factors (PDGF, FGF), interferons ( alpha, beta or...cells of an organ perfused by this vessel in the patient to express an exogenous **therapeutic** agent protein, wherein the protein **treats** the disease or may be useful for diagnostic purposes. The present method may be used to **treat** diseases, such as an ischemic disease, a vasomotor disease, **diabetes**, a malignancy, AIDS or a genetic disease.

The present method may use exogenous **therapeutic** agent proteins, such as tPA and modifications thereof, urokinase, streptokinase, acidic fibroblast growth factor, basic...

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#### ABSTRACT

The present invention relates to the production of large quantities of a novel chimeric **TGF- beta**, termed **TGF- beta** 1/ beta 2, by eucaryotic host cells transfected with recombinant DNA vectors containing the **TGF- beta** 1/ beta 2 precursor coding sequence controlled by expression regulatory elements. Simian **TGF- beta** 1 cDNA (Sharples et al., 1987, DNA 6:239-244) was modified so that the...

... encoding amino acid residue numbers 9-13, 17, 19, 25 and 26 of the mature **TGF- beta** 1 sequence were hanged to the nucleotides encoding the corresponding amino acids of the mature **TGF- beta** 2 structure. Simian codon usage was maintained. The chimeric **TGF- beta** 1/ beta 2 of the invention induces effects on the proliferation of vascular endothelial cells equivalent to those induced by **TGF- beta** 1.

... FIG. 1. Nucleotide and deduced amino acid sequence (SEQ ID No.: 1,2) of the **TGF- beta** 1/ beta 2 hybrid protein encoded by expression plasmid p5/dhfr.

FIG. 2A-2B Bioactivity...  
... as described in Section 6.1.3., infra. 2(B) Standard growth inhibition curve for **TGF- beta** 1.

FIG. 3. Immunoblot analysis of proteins secreted by 5 beta 41,2.5 cells ...

... and assayed by immunoblotting under nonreducing (lane 1) or reducing (lane 2) conditions with anti-**TGF- beta** 1 sub 369-381 as described in Section 6.1.5., infra.

FIG. 4A-4C. Effect of various **TGF- beta** s on vascular endothelial cells. Two-dimensional, five-day vascular cell cultures were

grown in DME plus 10% FCS (control) with additions of **TGF- beta 1**, **TGF- beta 2**, both at 0.5 ng/ml, or TGF-5 at concentrations of 0.05...

...BASMC;4(C) RFC. In all cases the TGF-5 beta hybrid molecule mimicked the **TGF- beta 1** isoform.

FIG. 5. Effect of various **TGF- beta** s on vascular cell migration. Migrating aortic endothelial and smooth muscle cells were treated with **TGF- beta 1** and **TGF- beta 2** at 0.5 ng/ml and TGF5 beta at concentrations of 0.05, 0.5 or 5.0 ng/ml. TGF-5 beta mimicked the **TGF- beta 1** response induced on BASMCs by increasing migratory rates, while the opposite effect was seen when using large vessel endothelial cells. **TGF- beta 2** elicited no effect on either cell type.

FIG. 6A-6F. Angiogenic response induced by various **TGF- beta** s. Three-dimensional RFC cultures were viewed with Hoffman interference microscopy to analyze angiogenic responses elicited by **TGF- beta 1**, **TGF- beta 2**, and TGF-5 beta. Four-day, 3-D small vessel endothelial cultures were cryosectioned...

...cultures 6(A) were grown in DME plus 10% FCS. Treated cultures included: 6(B) **TGF- beta 1** (0.5 ng/ml); 6(C) **TGF- beta 2** (0.5 ng/ml); and TGF-5 beta at 6(D) 0.05 ng/ml; 6(E) 0.5 ng/ml and (F) 5.0 ng/ml. **TGF- beta 1** evokes complex, branching tubular structures, while the control cultures showed minimal tube formation. TGF...

... 0.5 ng/ml of the hybrid molecule stimulated an angiogenic response equivalent to the **TGF- beta 1** isoform. The highest concentration of TGF-5 beta appears to cause a slight decrease in the amount of complex branching structures which had been shown before using **TGF- beta 1**. **TGF- beta 2** required a 10-fold increase in concentration (5.0 ng/ml) in order to match the neovascularization induced by **TGF- beta 1** and TGF-5 beta at 0.5 ng/ml. Arrows indicate tubular formations by...

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...1. INTRODUCTION

The present invention relates to a novel chimeric transforming growth factor-beta termed **TGF- beta 1/ beta 2** (or **TGF-5 beta**), to nucleotide sequences (SEQ ID No. 1) and expression vectors encoding **TGF- beta 1/ beta 2**, and to methods for the production of **TGF- beta 1/ beta 2**. The invention is exemplified by the production and secretion of **TGF- beta 1/ beta 2** by CHO cells transfected with expression vectors encoding a chimeric **TGF- beta 1/ beta 2** precursor gene (SEQ ID No. 1). The chimeric gene product possesses **TGF- beta** biological activity.

## 2. BACKGROUND OF THE INVENTION

Transforming growth factor-Beta (**TGF- beta**) is a member of a recently described family of polypeptides that regulate cellular differentiation and...

...gene complex of *Drosophila* (Padgett et al., 1987, *Nature* 325:81-84).

Four types of **TGF- beta** have been identified and designated **TGF- beta 1**, **TGF- beta 2**, **TGF- beta 1.2**, and **TGF- beta 3**. The first described type, **TGF- beta 1**, consists of two identical disulfide linked subunits having molecular weights of 13,000 (Assoian...

... *Biol. Chem.* 261:4377- 4379) and simian (Sharples et al., 1987, *DNA* 6:239-244) **TGF- beta 1** have been isolated. DNA sequence analysis of these clones indicates that **TGF- beta 1** is synthesized as a large precursor polypeptide, the carboxy terminus of which is cleaved to yield the mature **TGF- beta** monomer. Strong sequence homology has been found throughout the **TGF- beta 1** precursor protein from all of the above sources.

In the presence of 10% serum and epidermal growth factor, **TGF- beta 1** promotes the anchorage independent growth of normal rat kidney fibroblasts (Roberts et al., 1981 *Cancer Res.* 43:1518-1586). **TGF- beta 1** has also been shown to cause fetal rat muscle mesenchymal cells to differentiate and...

...1986, *J. Biol. Chem.* 261:5693-5695).

In contrast to its effect on cell proliferation, **TGF- beta 1** purified from human platelets has been shown to inhibit the growth of certain cells in culture (Tucker et al., 1984, *Science* 226:705-707). **TGF- beta 1** has also been shown to inhibit the growth of several human cancer cell lines...

... al., 1985, *Proc. Natl. Acad. Sci. USA* 82:119-123). This inhibitory/stimulatory effect of **TGF- beta 1** may depend on several factors including cell type and the physiological state of the cells (for review see Sporn et al., 1986, *Science* 233:532-534).

**TGF- beta 2**, like **TGF- beta 1**, is a polypeptide

of molecular weight 26,000 composed of two identical 13,000...

... Wrann et al., 1987, EMBO 6:1633-1636). cDNA clones coding for human and simian **TGF- beta 2** have been isolated (Madisen et al., 1988, DNA 7:1-8; Webb et al., 1988, DNA 7:493-497). The mature **TGF- beta 2** monomer is cleaved from one of two larger precursor polypeptides, the mRNAs of which may arise via differential splicing (Webb et al., 1988, DNA 7:493-497).

**TGF- beta 1** and **TGF- beta 2** share 71% amino acid sequence identity in their mature regions, and 41% identity in their precursor structures. **TGF- beta 3**, the amino acid sequence of which has very recently been deduced from cDNA clones...

...C-terminal 112 amino acid sequence with about 80% homology to the mature monomers of **TGF- beta 1** and **TGF- beta 2** (Dijke et al., 1988, Proc. Natl. Acad. Sci. USA 85:4715-4719). **TGF- beta 1.2** is a heterodimeric form comprising a beta 1 and beta 2 subunit linked ...

...disulfide bonds (Cheifetz et al., 1987, Cell 48:409-415).

## 2.1. INTRACELLULAR PROCESSING OF **TGF-.beta.1**

The amino portion of the precursor region of **TGF- beta 1** from human, rodent and simian sources show a high degree of homology (Derynck et ...

... associated with this part of the molecule. Recent studies demonstrating that this portion of the **TGF- beta 1** precursor is glycosylated and phosphorylated support this contention since one might assume that a...

... evidence which suggests that glycosylation of the precursor is involved in the transport of mature **TGF- beta 1** out of the cell (Purchio et al., 1988, J. Biol. Chem. 263:14211-4215...

... intermediate precursor complexes involved in processing or expression artifacts in CHO cells expressing the simian **TGF- beta 1** gene has been reported (Gentry et al., 1988, Mol. Cell. Biol. 8:4162-168...

... Gentry et al., 1987, Mol. Cell. Biol. 7:3418-427). These studies revealed that the **TGF- beta 1** precursor synthesized by transfected CHO cells consists of pro-**TGF- beta 1**, mature **TGF- beta 1**, and the pro region of the precursor interlinked by disulfide bonds. Such disulfide-linked precursor complexes have also been observed in isolated latent forms of **TGF- beta 1** (Miyazano et al., 1988, J. Cell. Biochem. Suppl. 12(A):200; Wakefield et al...Biol., 8:4162-4168) have proposed the following scheme for the processing of pre-pro-**TGF- beta 1** in transfected CHO cells. (The amino acid position numbers referred to are from the published sequence of simian **TGF- beta 1** (Sharples et al., 1987, DNA 6:239-244)). According to this proposed scheme, the...

... core glycosylation units (Rothman et al., 1978, Cell 15:1447-1454) are added to pro-**TGF- beta 1** at each of three predicted N-glycosylation sites located at Asn-82, Asn-136 and Asn-176. The core glycosylated pro-**TGF- beta 1** is then sequentially processed during transit through the Golgi to yield a phosphorylated glycoprotein...  
... synthesis or transit, proteolytic cleavage at the dibasic residue and disulfide isomerization occurs, releasing mature **TGF- beta 1**.

In another recent study, mannose-6-phosphate was identified in the **TGF- beta 1** precursor. Mannose-6-phosphate, a phosphorylated sugar analog, appears to play a fundamental role...

... Chem. 57:11145-11150). It is possible that the mannose-6-phosphate residues of the **TGF- beta 1** precursor may direct pro-**TGF- beta 1** to lysosomes for proteolytic processing to yield mature

**TGF- beta 1.** Alternatively, the mannose-6-phosphate residues may function to target the cleaved **TGF- beta 1** precursor to lysosomes for degradation.

### 3. SUMMARY OF THE INVENTION

The present invention relates to the production of large quantities of a novel chimeric **TGF- beta**, termed **TGF- beta 1/ beta 2**, by eucaryotic host cells transfected with recombinant DNA vectors containing the **TGF- beta 1/ beta 2** precursor coding sequence (SEQ ID No. 1) controlled by expression regulatory elements. Simian **TGF- beta 1** cDNA (Sharples et al., 1987, DNA 6:239-244) was modified so that the...

... encoding amino acid residue numbers 9-13, 17, 19, 25 and 26 of the mature **TGF- beta 1** sequence were changed to the nucleotides encoding the corresponding amino acids of the mature **TGF- beta 2** structure. Simian codon usage was maintained.

Expression vectors encoding the chimeric **TGF- beta 1/ beta 2** precursor under the regulatory control of Simian Virus 40 (SV 40) expression...

... Chinese Hamster ovary (CHO) cells. CHO transfectants which synthesize and secrete high levels of mature **TGF- beta 1/ beta 2** were obtained. **TGF- beta 1/ beta 2** expression was amplified with methotrexate and amplified transfectants secreted as much as 1 mg/L mature **TGF- beta 1/ beta 2**. Acidification of the conditioned media of the CHO transfectants resulted in maximal levels of bioactive **TGF- beta 1/ beta 2**. It is believed that the high levels of mature **TGF- beta 1/ beta 2** secreted by the transfected CHO cells results from an unusual efficiency in...

... processing efficiency may, in turn, result from structural characteristics affected by applicants' combination of the **TGF- beta 1** and **TGF- beta 2** amino acid sequences in the amino-terminal domain of the mature **TGF- beta** structure.

The chimeric **TGF- beta 1/ beta 2** of the invention induces effects on the proliferation of vascular endothelial cells equivalent to those induced by **TGF- beta 1** (see

### 5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to **TGF- beta 1/ beta 2**, to nucleotide sequences (SEQ ID No.: 1) encoding **TGF- beta 1/ beta 2** and the **TGF- beta 1/ beta 2** precursor, and to the production of **TGF- beta 1/ beta 2** by recombinant DNA methods. **TGF- beta 1/ beta 2**, a novel chimeric transforming growth factor-beta, is biologically active in the standard assay used to measure **TGF- beta 1** bioactivity and is immunoreactive with **TGF- beta 1**-specific antibodies. A chimera structurally comprising a combination of **TGF- beta 1** and **TGF- beta 2** amino acid sequences (SEQ ID No.: 2), the **TGF- beta 1/ beta 2** of the invention is likely to carry a novel portfolio of biological...

...nearly identical to those exhibited by its parent molecules while others may be unique to **TGF- beta 1/ beta 2**. With regard to those bioactivities which are similar or nearly identical to those of **TGF- beta 1** or **TGF- beta 2**, this new factor may provide a more effective means of inducing corresponding biological responses and its use may therefore be a desirable alternative to **TGF- beta 1** and **TGF- beta 2** in various medical applications envisioned for the **TGF- beta** s. Such applications include but are not limited to inducing or accelerating cell proliferation and differentiation and, inhibiting cell division. Thus **TGF- beta 1/ beta 2** may find uses in, for example, treating cancer and promoting wound healing...

... purposes of description: (a) generation of the coding sequence (SEQ ID No. 1) for the **TGF- beta 1/ beta 2** precursor; (b) construction of an expression vector which will direct the expression of the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No. 1); (c) transfection of appropriate host cells...

... replicating, expressing the gene and processing the gene product to produce the mature form of **TGF- beta 1/ beta 2** and/or **TGF- beta 1/ beta 2** precursors; and (d) identification and purification of the **TGF- beta 1/ beta 2** precursors and the mature, biologically active **TGF- beta 1/ beta 2**.

Once a transfectant is identified that expresses high levels of **TGF- beta 1/ beta 2** precursors and/or mature **TGF- beta 1/ beta 2**, the practice of the method of the invention involves the expansion of...

... The method of the invention is demonstrated herein, by way of examples in which simian **TGF- beta 1** precursor cDNA (Sharples et al., 1987, DNA 6:239-244) is modified so that...

...amino acid residue numbers 9-13, 17, 19, 25 and 26 of the mature simian **TGF- beta 1** sequence are changed to the nucleotides encoding the corresponding amino acids in the mature **TGF- beta 2** structure, while maintaining simian codon usage. The resulting chimeric **TGF- beta 1/ beta 2** precursor coding sequence (SEQ ID No. 1) is then used to construct expression vectors which are capable of directing the synthesis of the mature **TGF- beta 1/ beta 2** product.

The effects of **TGF- beta 1/ beta 2** on the proliferation, migration and angiogenesis of vascular endothelial cells have been examined in vitro (section 7., et seq.) and appear to closely parallel those induced by **TGF- beta 1** but not **TGF- beta 2**.

The various aspects of the method of the invention are described in more detail...

...the subsections below and in the examples that follow.

#### 5.1. GENERATION OF THE CHIMERIC **TGF-.beta.1/.beta.2** CODING SEQUENCE

The nucleotide coding sequence (SEQ ID No.: 1) for the chimeric **TGF- beta 1/ beta 2** is depicted in FIG. 1. In the practice of the method of...

... can be used to generate the recombinant molecules which will direct the expression of the **TGF- beta 1/ beta 2** product. Due to the degeneracy of the nucleotide coding sequences, other DNA...isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine.

The nucleotide sequence for simian **TGF- beta 1** may be obtained from simian cell sources (Sharples et al., 1989, DNA 6:239-244). The nucleotide sequence of the chimeric **TGF- beta 1/ beta 2** in FIG. 1 (SEQ ID No. 1) may be prepared by methods...

...in the art.

In a specific embodiment of the invention, the coding sequence for simian **TGF- beta 1** was obtained from a full length cDNA clone obtained from an African green monkey cell line, BSC-40 (Sharples et al., 1987, supra). The coding sequence of chimeric **TGF- beta 1/ beta 2** depicted in FIG. 1 (SEQ ID No. 1) was then derived from the simian **TGF- beta 1** cDNA by removing and replacing the coding sequences



of amino acid residue numbers 9, 10, 11, 12, 13, 17, 19, 25 and 26 of the mature **TGF- beta 1** molecule with the coding sequences for amino acid residue numbers 9, 10, 11, 12, 13, 17, 19, 25 and 26 of the mature **TGF- beta 2** molecule (Madisen et al., 1988, DNA 7:1-8) using gene construction techniques.

## 5.2. CONSTRUCTION OF EXPRESSION VECTORS CONTAINING THE CHIMERIC **TGF- beta.1/ beta.2** CODING SEQUENCE

In order to express biologically active, mature **TGF- beta 1/ beta 2**, an expression vector/host system should be chosen which provides not only...

... especially important when employing the entire coding sequence (SEQ ID No.: 1) of the chimeric **TGF- beta 1/ beta 2** precursor in the expression constructs because, like **TGF- beta 1** and **TGF- beta 2**, the mature chimeric **TGF- beta 1/ beta 2** is believed to be released from a precursor molecule or complex of...

...provides for secretion of the product may be desirable.

In particular, it appears that mature **TGF- beta 1/ beta 2** is a disulfide linked homodimer of 112 amino acids per subunit formed by cellular processing events believed to be similar to those which form mature **TGF- beta 1** and **TGF- beta 2**. The **TGF- beta 1/ beta 2** precursor has three potential N-glycosylation sites in its pro domain (Sharples et al., 1987, DNA 6:239-244). Studies involving **TGF- beta 1** have determined that N-glycosylation and phosphorylation in the pro domain of **TGF- beta 1** occurs in transfected CHO cells, implicating an important functional role for the precursor in...

...1988, Mol. Cell. Biol. 8:2229-2232). The presence of mannose-6-phosphate in the **TGF- beta 1** precursor also supports the hypothesis that the precursor has independent functional activity (Purchio et al., 1988, J. Biol. Chem. 263:14211-14215). Since the chimeric **TGF- beta 1/ beta 2** precursor contains the simian **TGF- beta 1** pro domain, applicants believe it likely that the **TGF- beta 1/ beta 2** precursor is functionally active and important to the correct processing the mature **TGF- beta 1/ beta 2** ...of a host cell used in the expression system to correctly express and process chimeric **TGF- beta 1/ beta 2** is important to the production of a mature, bioactive product.

In a specific embodiment described herein, mature bioactive **TGF- beta 1/ beta 2** is successfully produced using simian virus 40 (SV40) expression control elements in...

... e., vectors which contain the necessary elements for directing the replication, and translation of the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No.: 1) in an appropriate host cell) may...

... initiation codon and adjacent sequences. For example, in cases where only a portion of the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No.: 1) is inserted, exogenous translational control signals...

... provided. Furthermore, the initiation codon must be in phase with the reading frame of the **TGF- beta 1/ beta 2** coding sequences (SEQ ID No.: 1) to ensure translation of the entire...

... of DNA fragments into a vector may be used to construct expression vectors containing the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No.: 1) and appropriate transcriptional/translational control signals...

...recombinations (genetic recombination).

In cases where an adenovirus is used as an expression vector, the

**TGF- beta 1/ beta 2** coding sequence (SEQ ID No.: 1) may be ligated to an adenovirus...

... E3) will result in a recombinant virus that is viable and capable of expressing chimeric **TGF- beta 1/ beta 2** in infected hosts. Similarly, the vaccinia 7.5K promoter may be used.

An alternative expression system which could be used to express **TGF - beta 1/ beta 2** is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis...

... as a vector to express foreign genes. The virus grows in *Sodoptera frugiperda* cells. The **TGF- beta 1/ beta 2** coding sequence (SEQ ID No.: 1) may be cloned into non-essential...

...the control of an AcNPV promoter (for example, the polyhedrin promoter). Successful insertion of the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No.: 1) will result in inactivation of the...e.g. zinc and cadmium ions for metallothionein promoters). Therefore, expression of the genetically engineered **TGF- beta 1/ beta 2** may be controlled. This is important if the protein product of the...

...foreign protein expressed.

In a specific embodiment of the invention, an expression vector containing the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No. 1) in tandem with the mouse dihydrofolate...

... phenotype are isolated by propagation in selective media. To increase the level of expression of **TGF- beta 1/ beta 2**, transfectants may be exposed to increasing concentrations of methotrexate in order to isolate clones transcribing amplified levels of **TGF- beta 1/ beta 2** mRNA. **TGF- beta 1/ beta 2** mRNA levels may be assayed at various stages of amplification by solution...  
...Acad. Sci. U.S.A. 83:1300-1304).

### 5.3. IDENTIFICATION OF TRANSFECTANTS EXPRESSING CHIMERIC **TGF -beta.1/ beta.2**

The host cells which contain the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No. 1) and which express the biologically active...

... marker" gene functions; (c) assessing the level of transcription as measured by the expression of **TGF- beta 1/ beta 2** mRNA transcripts in the host cell; and (d) detection of the mature...

...immunoassay and, ultimately, by its biological activities.

In the first approach, the presence of the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No. 1) inserted in the expression vector can...

... detected by DNA-DNA hybridization using probes comprising nucleotide sequences that are homologous to the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No. 1) substantially as shown in FIG. 1...

... resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No. 1) is inserted within a marker gene sequence of the vector, recombinants containing the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No. 1) can be identified by the absence...

... the marker gene function. Alternatively, a marker gene can be placed in tandem with the **TGF- beta 1/ beta 2** sequence (SEQ ID No. 1) under the control of the same or different promoter used to control the expression of the **TGF- beta 1/ beta 2** coding sequence (SEQ ID

No. 1). Expression of the marker in response to induction or selection indicates expression of the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No. 1).

In the third approach, transcriptional activity for the **TGF- beta 1/ beta 2** coding region (SEQ ID No. 1) can be assessed by hybridization assays...

... RNA can be isolated and analyzed by Northern blot using a probe homologous to the **TGF- beta 1/ beta 2** coding sequence (SEQ ID No. 1) or particular portions thereof. Alternatively, total...of the success of the expression system, however, involves the detection of the biologically active **TGF- beta 1/ beta 2** gene product. Where the host cell secretes the gene product, the cell free media obtained from the cultured transfectant host cell may be assayed for **TGF- beta 1/ beta 2** activity. Where the gene product is not secreted, cell lysates may be...

...herein or the like may be used.

Once a clone producing high levels of mature **TGF- beta 1/ beta 2** is identified, the clone may be expanded and the **TGF- beta 1/ beta 2** may be purified using techniques well known in the art. Such methods...

...including high performance liquid chromatography, and the like.

#### 5.4. IMPROVED METHOD FOR PRODUCING MATURE **TGF-.beta 1/.beta.2**

In a specific embodiment of the invention, the **TGF- beta 1/ beta 2** precursor may be modified by eliminating the amino acid cysteine at position...

... at the DNA level using site directed mutagenesis. COS cells transfected with plasmids encoding the **TGF- beta 1** precursor gene so modified ultimately secrete between three and five times more mature **TGF- beta 1** than do CHO cells expressing the unmodified precursor gene. It is believed that this...

... complexes in the transfected COS cells and therefore allows for the secretion of more mature **TGF- beta**.

#### 6. EXAMPLE: PRODUCTION OF **TGF-.beta.1/.beta.2** BY EXPRESSION IN CHINESE HAMSTER OVARY CELLS

A recombinant plasmid encoding **TGF- beta 1** precursor in which amino acids 9, 10, 11, 12, 13, 17, 19, 25 and 26 of the mature **TGF- beta 1** sequence were replaced by the corresponding amino acids of the mature **TGF- beta 2** sequence was constructed (SEQ ID No. 1). Specifically, amino acid 9 of mature **TGF- beta 1** (serine) was replaced by arginine, amino acid number 10 (serine) was replaced by asparagine...

... was used to transfect CHO cells. Transfectants which produced and secreted a mature, bioactive, chimeric **TGF- beta 1/ beta 2** were isolated.

#### 6.1. MATERIALS AND METHODS

6.1.1. DNA TRANSFECTIONS...1 Lu (Accession Number CCL-64, American Type Culture Collection), which are extremely sensitive to **TGF- beta** were utilized for the growth inhibition assay. The assay was performed using the thymidine analog...

...IdU compared to untreated CCL-64 cells.

To assay transfected cells for secretion of active **TGF- beta** 1/ beta 2, serum free supernatants were collected from one 24 hour collection on confluent...

...2-3 week intervals. Bleedings were taken 7-14 days following the boosts.

Anti-peptide **antibodies** directed toward peptide sequences within the **TGF- beta** 1 molecule were generated in rabbits using synthetic peptides as immunogens (Gentry et al., 1987, Mol. Cell. Biol. 7:3418-3427). One of the **antibodies** (anti-**TGF- beta** 1 sub 369-381) was directed toward epitopes present within the mature form of the **TGF- beta** growth factor. The other two **antibodies** (anti-**TGF- beta** 1 sub 81-94 and anti-**TGF- beta** 1 sub 225-236) are precursor-specific and are directed toward peptide sequences (SEQ ID No. 2) present only within the precursor molecule of **TGF- beta** 1.

#### 6.1.5. IMMUNOBLOTTING

Proteins were fractionated on 7.5%-17.5% gradient SDS... S.A. 80:4045-4049).

#### 6.1.6. CONSTRUCTION OF PLASMID PROGRAMMING THE SYNTHESIS OF **TGF-.beta.1/.beta.2**

The plasmid programming the synthesis of the chimeric **TGF- beta** 1/ beta 2 protein, p5 beta /dhfr, was constructed as follows. pAc beta **TGF- beta** 1, a baculovirus vector derived from pAc373 (Miyamoto et al., 1985, Cell. Biol. 5:2860...

... Virology 158:248-250), which contains the 1.4 Kb PstI-EcoRI coding sequence of **TGF- beta** 1 (Sharples et al., 1987, DNA 6:239-244) cloned into the PstI-EcoRI site...

... 248-250), was digested with BamHI and EcoRI and the 375 bp fragment of the **TGF- beta** 1 coding sequence was isolated (Fragment 1). pSV2-TGF (Gentry et al., 1987, Mol. Cell...

... with Klenow enzyme, digested with HindIII and the 1.4 Kb fragment containing the chimeric **TGF- beta** 1/ beta 2 coding sequence (SEQ ID No.: 1) was isolated (Fragment 3). 5 beta...

... p5 beta /dhfr was isolated. The nucleotide and deduced amino acid sequences of the chimeric **TGF- beta** 1/ beta 2 molecule encoded by p5/dhfr are shown in FIG. 1 (SEQ ID No. 1, 2, 4).

#### 6.2. EXPRESSION OF **TGF-.beta.1/.beta.2** IN CHO CELLS

p5 beta /dhfr was transfected into CHO cells and...

... CHO-5 beta 41,2.5 cells secrete approximately 1 mg/L of bioactive chimeric **TGF- beta** 1/ beta 2 (FIG. 2).

**TGF- beta** related proteins secreted by these cells were analyzed by immunoblotting using anti-peptide **antibodies** directed against mature **TGF- beta** 1 as described in Section 6.1.5., supra FIG. 3 shows that CHO-5...

... PAGE under nonreducing conditions (FIG. 3, lane 1). The 24 kilodalton band represents the mature **TGF- beta** 1/ beta 2 dimer and the 90 to 100 kilodalton protein probably represents mature **TGF- beta** 1/ beta 2 disulfide-bonded to precursor sequences (Gentry et al., 1987, Mol. Cell. Biol. migrate at 12 kilodaltons, representing the mature **TGF- beta** 1/ beta 2 monomer. Note the lack of immunoreactive material in the 45 to 55...

... similar analysis of recombinant proteins expressed in CHO cells

transfected with plasmids encoding the simian **TGF- beta 1** gene (Gentry et al., 1987, Mol. Cell. Biol. 7:3418-3427) suggesting that chimeric **TGF- beta 1/ beta 2** is proteolytically processed more efficiently than its parent molecule **TGF- beta 1**. In addition, CHO-5 **beta 41,2.5** cells secrete about 2.5 times more bioactive mature product than do CHO cells expressing **TGF- beta 1** (Gentry et al., 1987, supra). Although the basis for these observations is presently unknown, the secondary structure of the chimeric **TGF- beta 1/ beta 2** precursor may significantly differ from the secondary structure of **TGF- beta 1**, which secondary structure renders the chimeric **TGF- beta 1/ beta 2** subject to molecular processing events of a different intensity or nature. For example, the **TGF- beta 1/ beta 2** precursor may be a more favorable substrate for the factors involved in **TGF- beta** processing. Alternatively, the secondary structural characteristics of **TGF- beta 1/ beta 2** may allow it to interact with other processing factors or pathways not as accessible to **TGF- beta 1**.

#### 7. EXAMPLE: GROWTH MODULATION OF VASCULAR ENDOTHELIAL CELLS BY HYBRID **TGF-5.beta.**

Using three distinct bioassays which previously indicated that differential responses are induced by **TGF- beta 1** and **TGF- beta 2** (Merwin et al., 1991, Am. J. Path. 138(1):37-51), the biological effects...

... microvascular endothelial cells; the results were equivalent to those obtained with RFCs.

##### 7.1.2. **TGF-.beta.** FACTORS

Recombinant hybrid **TGF- beta 1/ beta 2** was produced as described in Section 6, et seq, supra. **TGF- beta 1** and **TGF- beta 2** were prepared, respectively, as described in Assoian et al., 1983, J. Biol. Chem. 258...

... cells on the substrate was determined. At this time point, fresh medium with or without **TGF- beta s** was added to the cultures. Medium and **TGF- beta s** were replaced once again on day 3. Cell numbers were determined by ...counter (Coulter Electronics, Inc., Hialeah, Fla.). The mean number of cells per dish for each **TGF- beta** addition was then calculated.

##### 7.1.5. CELL MIGRATION ASSAYS

Stimulus for cell migration was...

... in a radial fashion. Cultures were fed once again on day 3 with control or **TGF- beta** -supplemented media. After 6 days, the cultures were washed with PBS, fixed with 10% neutral...

...Univeristy, New Haven, Conn.).

#### 7.2. RESULTS

The majority of published reports indicate that the **TGF- beta 1** and **TGF- beta 2** isoforms are equipotent in vitro. However, recent reports indicate that these isoforms are not...

... Science 239:783-85; and Danielpour et al., 1989, J. Cell Physiol. 138:79-86). **TGF- beta 1** is reportedly more effective than **TGF- beta 2** in inhibiting DNA synthesis in endothelial cells (Jennings, et al., 1988, J. Cell. Physiol. 137:167-72).

A recent report establishes that **TGF- beta 1** and **TGF- beta 2** isoforms elicit differential responses in proliferation assays using three vascular cell types (Merwin et...

... the effects of TGF-5 beta on endothelial cells seemed ideal for determining whether the **TGF- beta 1/TGF- beta 2** hybrid (TGF-5 beta) would evoke responses similar to **TGF- beta 1** or to **TGF- beta 2**. The results indicated that TGF-5 beta mimicked **TGF- beta 1** in all three proliferation assays examined, while **TGF- beta 2** displayed unique properties.

Both **TGF- beta 1** and TGF-5 beta inhibited BAEC proliferation by more than 70% (+/-1.5%) at the optimum concentration of 0.5 ng/ml (FIG. 4A). In contrast, **TGF- beta 2** was only able to evoke inhibition of about 20% (+/-2.5%). When using BASMCs...

... 4B). Microvascular endothelial cells were growth inhibited as much as 70% (+/-4.0%) by both **TGF- beta 1** and TGF-5at concentrations of 0.5 ng/ml (FIG. 4C). At equivalent concentration, however, **TGF- beta 2** was only able to mount a 37% (+/-8%) inhibition; however, with a 10-fold increase in concentration, **TGF- beta 2** was able to evoke a similar response.

Similarly, hybrid TGF-5 beta exerted a cell migration response equivalent to that induced by **TGF- beta 1**. The results presented in FIG. 5 show that BAECs are inhibited by both TGF-5 beta and **TGF- beta 1**, while BASMCs are growth-stimulated by both factors. In contrast, **TGF- beta 2** had no effect on the migration of either cell type.

The above results may...

... variety of molecules. In this regard, one of the major factors released by platelets is **TGF- beta 1**. With a marked increase in the local concentration of **TGF- beta 1**, endothelial cells may be inhibited from migrating to affect a rapid re-endothelialization of...tumor growth. Therefore, understanding the enhancement of neovascularization is of paramount concern. To investigate the **TGF- beta** -induced angiogenic response in microvascular endothelial cells, RFCs were grown in three-dimensional collagen cultures for 4 days, either in the presence or absence of **TGF- beta** isoforms. All three isoforms were able to stimulate RFCs to mount a neovascularization response. This...

... metabolic stimulation (increased numbers of ribosomes and engorged ER), cellular polarity, deposition and organization of **extracellular matrix** proteins and lumen formation.

FIG. 6A shows control cultures with no exogenous growth factor. Cells remain independent of one another and lack tubular structures, while **treatment** with **TGF- beta 1** (FIG. 6B) reveals complex, tube-like formations. **TGF- beta 2** (FIG. 6C) was able to initiate a modest angiogenic response. However, as with RFC proliferation studies, an increase in **TGF- beta 2** concentration from 0.5 to 5.0 ng/ml was necessary for the establishment of complex tubular structures. TGF-5 beta mimicked **TGF- beta 1**, with tube formation initiated with as little as 0.05 ng/ml (FIG. 6D...

...highest concentration used, 5.0 ng/ml (FIG. 6F).

In summary, TGF-5 beta mimics **TGF- beta 1** in inhibiting proliferation of low density monolayer micro and large vessel endothelial cell cultures by about 70% and by about 45% on BASMCs. In contrast, an equivalent concentration of **TGF- beta 2** is ineffective with BAECs and equivalent on BASMCs. Moreover, a 10-fold increase in **TGF- beta 2** concentration is required to elicit a similar response with RFCs. When using a culture...

... begins with a tight, confluent monolayer, response induced by the isoforms differ. In particular, both **TGF- beta 1** and **TGF- beta 2** induce equivalent levels of BASMC migration enhancement and

BAEC migration inhibition, while TGF- beta 2 does not influence the migration of either cell type. Finally, in microvascular endothelial cells which respond to injury in vivo by neovascularization, all three TGF- beta s were able to stimulate angiogenesis in vitro. However, a 10-fold increase in TGF- beta 2 concentration was necessary to match the level of response induced by TGF- beta 1 and TGF-5 beta.

#### 8. DEPOSIT OF MICROORGANISMS

The following transfectant has been deposited...

2/K/85 (Item 85 from file: 654)  
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#### ABSTRACT

A polypeptide is provided that excludes (a) a full-length mature TGF- beta molecule or precursor TGF- beta molecule or deletion variants of mature or precursor TGF- beta molecules in which from about 1 to 10 amino acid residues have been deleted, (b...

... comprising an amino acid sequence that is based on conserved sequences in the family of TGF- beta molecules. Such polypeptides are particularly useful therapeutically as immunosuppressive agents when coupled to carrier proteins...

... the CCL-64 mink lung cell proliferation assay versus the reciprocal of dilution of a TGF- beta polypeptide conjugated to HSA (black), HSA control (hatched), and the unconjugated polypeptide (checked).

FIG. 2...

... amount of PGE2 released by IL-1 treated human fibroblasts as a function of various TGF- beta peptides, TGF- beta 1, HSA alone, and cells alone.

FIG. 3 represents a graph of the percent radiolabeled TGF- beta 1 bound to its receptors as a function of the concentration of TGF- beta peptide dimer present.

FIG. 4 represents a bar graph of percent inhibition of tetanus-toxoid...

... circles with lines are FMLP 10 sup -9 M, and squares without lines are the TGF- beta peptide response).

... U.S. application Ser. No. 07/356,963 filed concurrently herewith, entitled "Nucleic Acid Encoding TGF- beta Variants and Their Uses."

#### BACKGROUND OF THE INVENTION

##### 1. Field of the Invention

This invention...

...infections, and to immunodiagnostic reagents.

##### 2. Description of Related Art

The transforming growth factor- beta (TGF- beta) molecules identified thus far are each dimers containing two identical 112 residue polypeptide chains linked by disulfide bonds. The molecular mass of these dimers is about 25 kd. Biologically active TGF- beta has been defined as a molecule capable of inducing anchorage independent growth of target cell...

... in vitro cell culture, when added together with EGF or TGF- alpha as a cofactor. TGF- beta is secreted by virtually all cell types in

an inactive form. This latent form can be activated by proteolytic cleavage of mature **TGF- beta** from its precursor (at the Arg-Ala bond in position 278). A non-covalent complex is formed from the association of the mature **TGF- beta** with the precursor remainder or with a protein binding to **TGF- beta** or with alpha sub 2 -macroglobulin. This complex is disrupted so as to activate the **TGF- beta** either by exposure to transient acidification or by the action of exogenous proteases such as plasmin or plasminogen activator.

There are at least three forms of **TGF- beta** currently identified, **TGF- beta** sub 1, **TGF- beta** sub 2, and **TGF- beta** sub 3. Suitable methods are known for purifying this family of **TGF- beta** s from various species such as human, mouse, green monkey, pig, bovine, and chick, and...

... 85: 79-82 (1988), the entire contents of these publications being expressly incorporated by reference.

**TGF- beta** has been shown to have numerous regulatory actions on a wide variety of both normal and neoplastic cells. Recent studies indicate an important role for **TGF- beta** in cells of the immune system (J. Kehrl et al., J. Exp. Med. 163:1037...S.A., 83:2438 [1986]; G. Shipley et al. Cancer Res. 46:2068 [1986]). Moreover, **TGF- beta** has been described as a suppressor of cytokine (e.g., IFN- gamma, TNF- alpha) production...

... a promoter of cachexia (Beutler and Cerami, New Eng. J. Med., 316: 379 [1987]). Further, **TGF- beta** induces collagen secretion in human fibroblast cultures (Roberts et al., Proc. Nat. Acad. Sci. USA...

... 82:4535 [1985]); and inhibits endothelial regeneration (R. Heimark et al., Science 233:1078 [1986] ).

**TGF- beta** is multifunctional, as it can either stimulate or inhibit cell proliferation, differentiation, and other critical processes in cell function (M. Sporn, Science. 233:532 [1986]).

The multifunctional activity of **TGF- beta** is modulated by the influence of other growth factors present together with the **TGF- beta**. **TGF- beta** can function as either an inhibitor or an enhancer of anchorage-independent growth, depending on...

...of growth factors, e.g., EGF or **TGF- alpha**, operant in the cell together with **TGF- beta** (Roberts et al., Proc. Natl. Acad. Sci. U.S A., 82:119 [1985]). **TGF- beta** also can act in concert with EGF to cause proliferation and piling up of normal...

... not rheumatoid) synovial cells (Brinkerhoff et al., Arthritis and Rheumatism. 26:1370 [1983]).

Most recently, **TGF- beta** has been found to suppress the expression of Class II histocompatibility antigens on human cells...

...17-19, 1987]; Palladino et al., Immunobiology, 175: 42 [1987]).

For a general review of **TGF- beta** and its actions, see Sporn et al., J. Cell Biol., 105: 1039-1045 (1987) and Sporn and Roberts, Nature, 332: 217-219 (1988).

Fragments of **TGF- beta** are described in EP 290,012 published 11/9/88 and EP 267,463 published 5/18/88. The former describes fragments of **TGF- beta** 2 having at least about eight amino acids, for example, in the region of N...

... patent publication describes in claim 36 a 20-mer consisting of an internal sequence of **TGF- beta** 3.



TGF- alpha polypeptides that compete with the EGF receptor are disclosed in U.S... to provide a novel class of immune modulators that are useful as immunogens to elicit **antibodies** to TGF- **beta**, i.e., TGF- **beta** antagonists.

It is another object to provide a novel class of immune modulators that are...

... to develop diagnostic assays for the presence in patient fluids of immunosuppressive proteins such as TGF- **beta**, or **antibodies** to such proteins.

These and other objects will become apparent to those skilled in the...

... that, if greater than 15 residues does not have the sequence of mature or precursor TGF- **beta** at a homologous location in the mature or precursor TGF- **beta** molecule;  
B is Leu, Asn, Ala, Pro, Arg, or Gln;  
C is Arg or Leu...

... that, if greater than 15 residues, does not have the sequence of mature or precursor TGF- **beta** at a homologous location in the mature or precursor TGF- **beta** molecule; and physiologically acceptable salts or esters thereof; provided, however, that the polypeptide excludes (a) a full-length mature TGF- **beta** molecule or precursor TGF- **beta** molecule or deletion variants of mature or precursor TGF- **beta** molecules in which from about 1 to 10 amino acid residues have been deleted (b...

... that, if greater than 15 residues, does not have the sequence of mature or precursor TGF- **beta** at a homologous location in the mature or precursor TGF- **beta** molecule; A is Val, Leu, Ala, or Tyr; and B-K are defined above.

In... with the polypeptide identified above that has the sequence containing the X moiety and isolating **antibodies** generated by the polypeptide that neutralize at least one immunosuppressive protein, preferably TGF- **beta**.

In yet another embodiment, the invention provides a monoclonal **antibody** specific for the polypeptide identified above and its various labeled and immobilized forms.

In another...

... as to form a polymer of the monomeric polypeptide. Preferably the monomeric polypeptide is the TGF- **beta** peptide described herein.

A novel class of polypeptides and analogs thereof have been discovered that...

... or part thereof, which is strikingly well conserved among a variety of proteins in the TGF- **beta** family. These proteins include TGF- **beta** 1, TGF- **beta** 2, TGF- **beta** 3, the inhibin alpha and beta chains, decapentaplegic protein (dpp), and the bone morphogenic proteins...  
DESCRIPTION OF THE PREFERRED EMBODIMENTS

#### A. Definitions

As used herein, the terms "mature TGF- **beta**," "TGF- **beta**," and "precursor TGF- **beta** " refer to the family of molecules described hereinabove that have either the full-length, native amino acid sequence of any of the TGF- **beta** s from any species but lacking the signal sequence ("mature") or the full-length, native amino

acid sequence of any of the **TGF- beta** s from any species with the naturally occurring signal sequence, including the latent forms and associated or unassociated complex of precursor and mature **TGF- beta** ("precursor"). Reference to such full-length **TGF- beta** herein will be understood to be a reference to any one of the currently identified forms, including **TGF- beta** sub 1, **TGF- beta** sub 2 and **TGF- beta** sub 3.

The sequence "Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys...

...Leu-Tyr-Ile-Asp-Phe-Arg-Gln-Asp-Leu" is the 20-mer derived from **TGF- beta** 3 that is described in EP 267,463, supra.

As used herein, the term "carrier...

... immunosuppressive activity. For example, a polypeptide representing an internal, active sequence within the full-length **TGF- beta** sequence would have **TGF- beta** as its immunosuppressive protein to be neutralized.

As used herein, the term "inflammatory disorder" refers...are defined as those having the ability to cross-react with antisera raised against native **TGF- beta** (where native **TGF- beta** is that which is obtained from platelets or other natural sources). Immunological cross-reactivity is...their N- or C-terminus, they do not have the sequence of mature or precursor **TGF- beta** at an analogous (homologous) position in the corresponding native mature or precursor **TGF- beta** molecule. This language thus excludes polypeptide fragments of native **TGF- beta** that may have been obtained incidentally by random digestion of the native molecule with a...

...L-M-N, as described above. More preferably, the 16-mer is derived from a **TGF- beta**, and comprises the sequence:Cys-A-Arg-B-Leu-Tyr-Ile-A sp-Phe-H-I...

... example, an indication of biological activity is whether the monomeric peptide neutralizes monoclonal and polyclonal **antibodies** raised against a corresponding native protein known to have biological/therapeutic activity, e.g., mature human **TGF- beta**. Other indications, specific for the **TGF- beta** polypeptide herein, include whether the peptide stimulates release of PGE2 by IL-1 treated human fibroblasts, interferes with the binding of full-length **TGF- beta** to its receptors, or acts as an immunosuppressive agent either in vitro or in vivo ... also are useful when administered by bronchial lavage or intravenous injection to patients with adult **respiratory distress** syndrome or septic shock.

It is within the scope hereof to combine the polypeptide polymer **therapy** with other novel or conventional **therapies** (e.g., growth factors such as EGF or **TGF- alpha**) for the disorders in question...

... are, in general, related to immunosuppressive proteins, they are also useful as immunogens to elicit **antibodies** capable of blocking the immunosuppressive activity associated with or caused by such immunosuppressive proteins, e.g., **TGF- beta**. Examples of such activity include neoplastic or viral disorders. For making immunogenic peptides capable of eliciting **antibodies** to the immunosuppressive proteins, the polypeptides are typically not immunosuppressive, either because they are in... ATCC CCL 64, Rockville, Md.), a mink lung epithelial-like cell, was used to detect **TGF- beta** activity. Mul-1-Lv cells maintained by weekly passage in vitro in CMEM consisting of ...

... counted in a liquid scintillation counter (LS 6800, Beckman, Fullerton, Calif.). Results (pg/ml of **TGF- beta**) were calculated based on percent inhibition of thymidine incorporation compared with a rHu**TGF- beta** sub...

...1 shows the cpm incorporated as a function of the reciprocal of dilution for the **TGF- beta** peptide conjugated to HSA (black), the HSA control (slashes), and unconjugated **TGF- beta** peptide (checks). The activities of both the free polypeptide and the HSA-conjugated polypeptide were... the designations indicated as follows, from left to right in the graph in the figure: **TGF- beta**, 250 ng/ml (solid bar), **TGF- beta** peptide conjugated to HSA diluted 1:10 (dark diagonal bar), **TGF- beta** peptide conjugated to HSA diluted 1:20 (light crosshatched bar), HSA alone diluted 1:10...

... bar), HSA diluted 1:20 (white bar), **TGF- peptide**, 100 mu M (dark crosshatched bar), **TGF- beta** peptide, 50 mu M (horizontally lined bar), **TGF- beta** peptide dimer, 100 mu M (medium dotted bar) **TGF- beta** peptide dimer, 50 mu M (thin diagonal lines on white background bar), and cells alone as control (light dotted bar). The results show that the conjugated **TGF- beta** peptides and peptide dimers are the most active in this bioassay to stimulate PGE sub 2 production from fibroblasts. The HSA control and cells alone were inactive.

### 3. **TGF- beta** Radioreceptor Binding Assay (FIG. 3)

The appropriate number of 24-well tissue culture plates were...5 ml assay diluent. A total of 10 mu l of recombinant full-length mature **TGF- beta** in 4 mM HCl and 1% w/v BSA was pipetted into tube NSB. A different lot of full-length mature **TGF- beta** in 4mM HCl and 1% w/v BSA (standard stock) was diluted in accordance with the following schedule, where "standard stock I" is obtained by diluting the **TGF- beta** standard stock 1:5 with assay diluent (i.e., 20 mu l standard stock and

... that, if greater than 15 residues, does not have the sequence of mature or precursor **TGF- beta** at a homologous location in the mature or precursor **TGF- beta** molecule;

A is Val or Leu;  
B is Pro or Gln;  
H is Arg or...

... salt or ester thereof; provided, however, that the polypeptide excludes (a) a full-length mature **TGF- beta** molecule or precursor **TGF- beta** molecule or deletion variants of mature or precursor **TGF- beta** molecules in which from about 1 to 10 amino acid residues have been deleted, (b)...

2/K/86 (Item 86 from file: 654)  
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### ABSTRACT

... Preferably, the transforming growth factor-beta is human transforming growth factor-beta, and most preferably **TGF- beta** sub 1, **TGF- beta** sub 2, or **TGF- beta** sub 3. ... 1a and 1b show the cDNA sequences and deduced amino acid sequences of porcine and human **TGF- beta** sub 3, respectively. The 112 amino acid sequence of mature **TGF- beta** sub 3 (overlined) constitutes the C-terminus of the precursor and is preceded by four...

... the homology between the imputed amino acid sequences of the human (h) and porcine (p) **TGF- beta** sub 3 precursors. The asterisks mark identical residues, while a dot indicates a conservative amino acid replacement. The mature **TGF- beta** sub 3 sequences are boxed. FIG. 2 shows the N-termini for selected forms of **TGF- beta**. The letters h, p and b stand for human, porcine and bovine, respectively. Non-homologous...

... 3 shows the percent cumulative lethality of mice over time upon treatment with recombinant human **TGF- beta** or recombinant human

IL-1 alpha versus the PBS control at various doses and times...  
... 3 on the percent cumulative lethality of mice over time upon treatment with recombinant human **TGF- beta** versus the PBS control at various doses and times relative to challenge with endotoxin.

FIG. 5 shows the percent cumulative lethality upon treatment of mice with recombinant human **TGF- beta** or recombinant human IL-1 alpha versus the PBS control after cecal ligation and puncture...  
... by disulfide bonds. The molecular mass of these dimers is about 25 kd. Biologically active **TGF- beta** has been defined as a molecule capable of inducing anchorage independent growth of target cell...

... with EGF or TGF- alpha as a co-factor. Suitable methods are known for purifying **TGF- beta** from platelets or placenta, for producing it in recombinant cell culture and for determining its...

...al, Cell 48: 409-415 (1987), the entire contents thereof being expressly incorporated by reference.

**TGF- beta** has been shown to have numerous regulatory actions on a wide variety of both normal and neoplastic cells. Recent studies indicate an important role for **TGF- beta** in cells of the immune system (J. Kehrl et al., J. Exp. Med., 163:1037...

... A., 83:2438 [1986] and G. Shipley et al. Cancer Res., 46:2068 [1986]]. Moreover, **TGF- beta** has been described as a suppressor of cytokine (e.g., TNF- alpha) production (Espevik et...

... as a promoter of cachexia (Beutler and Ceramie, New Eng. J. Med., 316: 379ff [1987]).

**TGF- beta** is multifunctional, since it can either stimulate or inhibit cell proliferation, can either stimulate or...

...critical processes in cell function (M. Sporn, Science 233:532 [1986]).

The multifunctional activity of **TGF- beta** is modulated by the influence of other growth factors present together with the **TGF- beta**. **TGF- beta** can function as either an inhibitor or an enhancer of anchorage-independent growth, depending on...

...of growth factors, e.g., EGF or TGF- alpha, operant in the cell together with **TGF- beta** (Roberts et al., Proc. Natl. Acad. Sci. U.S.A., 82:119 [1985]). According to Brinkerhoff et al., Arthritis and Rheumatism, 26:1370 (1983), **TGF- beta** can act in concert with EGF to cause proliferation and piling up of normal (but...

... synovial cells. Furthermore, Chua et al., J. Biol. Chem., 260:5213-5216 [1983] reported that **TGF- beta** induced collagenase secretion in human fibroblast cultures, and A. Tashjian et al., Proc. Natl. Acad. Sci. U.S.A., 82:4535 [1985] observed that **TGF- beta** stimulated the release of prostaglandins and mobilization of calcium. **TGF- beta** also has been reported to inhibit endothelial regeneration (R. Heimark et al., Science, 233:1078 [1986]).

U.S.S.N. 500,833, supra, relates to the use of **TGF- beta** to repair tissue in animals, in particular for use in accelerating wound healing by stimulating...

...U.S. Pat. Nos. 4,810,691 and 4,774,228 describe the use of **TGF- beta** for promoting connective tissue deposition.

U.S. Ser. No. 07/116,101 filed Nov. 3 disclose use of **TGF- beta** as an immunosuppressant, to treat inflammatory diseases such as rheumatoid arthritis.

It has been found...

... or at risk of septic shock a pharmaceutically effective amount of transforming growth factor-beta (TGF- beta).

In another aspect, this invention provides a composition for the treatment or prevention of septic shock comprising a pharmaceutically effective amount of transforming growth factor-beta (TGF- beta) and a pharmaceutically effective amount of a substance selected from the group consisting of mafenide...  
...produce different symptoms in the patient.

At the present time five highly homologous forms of TGF- beta have been identified, TGF- beta sub 1, TGF- beta sub 2, TGF- beta sub 3, TGF- beta sub 4, and TGF- beta sub 5. 304 N-termini for the first three of these forms are set forth in FIG. 2. Reference to TGF- beta herein will be understood as reference to any one of these identified forms as well...

...in the method described herein.

As can be seen from FIGS. 1a-1c, the mature TGF- beta sub 3 amino acid sequence contains a large number of cysteine residues, at least some of which apparently are involved in interchain crosslinking in forming the homodimeric TGF- beta that is recovered from natural sources. The rest of the precursor contains only two cysteine residues. The complete TGF- beta sub 3 precursor contains several pairs of basic residues that could also undergo post-translation cleavage and give rise to separate polypeptide entities.

Comparison of the porcine and human TGF- beta sub 3 precursor sequences (see FIG. 1c) reveals a 90% amino acid identity. The amino...

... C-terminal 112 amino acid sequence has about 80% similarity to the porcine and human TGF- beta sub 1 sequence and shares a similar degree of homology with the sequence of TGF- beta sub 2 .

In accordance with the method of this invention, the TGF- beta . is administered prophylactically or therapeutically, i.e., before, simultaneous with, or after the infection has set in. The TGF - beta may be used passively to treat individuals who suffer from septicemia or are at risk...

...endobronchitic infection endemic in cystic fibrosis patients.

It is within the scope hereof to employ TGF- beta from animals other than humans, for example, porcine or bovine sources, to treat humans. Likewise...

... other mammalian species such as domestic and farm animals and sports or pet animals, human TGF- beta, as well as TGF- beta from other species, is suitably employed. In one instance of animal treatment, dairy cows are treated for acute coliform mastitis infections by using TGF- beta to remove and neutralize the effects of endotoxin. Thus, the term "patient" as used herein refers to all mammals, not just humans.

The TGF- beta is administered to the patient by any suitable technique, including parenteral and, if desired for...

...is therapeutic or prophylactic. Thus, in view of the therapeutic urgency attendant frank shock, the TGF-beta is preferably intravenously infused at the same time as solutions used for initial volume expansion and intraperitoneal administration, preferably intravenous or intraperitoneal.

The **TGF- beta** compositions to be used in the therapy will be formulated and dosed in a fashion...

... the clinical condition of the individual patient, the cause of the septic shock, whether the **TGF- beta** is used for therapy of frank shock or prophylaxis of incipient septic shock, the site of delivery of the **TGF- beta**, the method of administration, the scheduling of administration, and other factors known to practitioners. The...

...determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the **TGF- beta** administered parenterally per dose will be in the range of approximately 1 mu g/kg...

... per day, although, as noted above, this will be subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained. Relatively higher doses may be needed initially for the treatment of profound shock, i.e., for patients in acute renal failure or respiratory distress, or having severely depressed blood pressure (mean arterial pressure below about 60 mm Hg).

The **TGF- beta** is used in an activated form as well as in latent forms for slow-release formulations. Preferably, the **TGF- beta** is activated, as by such methods as exposure to acidic or basic pH values, sodium...

... 6407-6415 (1988), the disclosure of which is incorporated herein by reference. For example, the **TGF- beta** may be treated with acid to give activity at pH below 6, preferably below 5.5, or incubated with 0.02% SDS or 8 M urea.

For parenteral administration, the **TGF- beta** is formulated generally by mixing it at the desired degree of purity, in a unit...

... acids, carbohydrates including glucose or dextrans, chelating agents such as EDTA, or other excipients. The **TGF- beta** is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml at pH range 4 to 6.

**TGF- beta** for use in therapeutic administration must be sterile. This is readily accomplished by sterile filtration through (0.2 micron) membranes. **TGF- beta** ordinarily will be stored as an aqueous solution since it is highly stable to thermal and oxidative denaturation although lyophilized formulations for reconstitution are acceptable.

**TGF- beta** therapy or prophylaxis is suitably combined with other proposed or conventional therapies or prophylactic treatment for septic shock. For example, for treatment of burns, the **TGF- beta** therapy may be delivered by separate means, simultaneously with and by the same administration route...

... inhibit bacterial colonization of the burn wound surface. Other therapies that can be combined with **TGF beta** therapy include primary therapeutic agents, for example potent anti-microbial agents such as aminoglycosides (such...

... Various adjunctive agents in the treatment of septic shock also are useful in combination with **TGF- beta**. These include sympathomimetic amines (vasopressors) such as norepinephrine, epinephrine, isoproterenol, dopamine, and dobutamine; antiinflammatory agents... incorporated herein by reference.

Therapeutic measures that can be used in conjunction with administering the **TGF- beta** include granulocyte transfusion and percutaneous

drainage of abdominal abscesses. In addition, prophylactic measures that are useful in conjunction with the **TGF- beta** involve, e.g., barrier isolation to minimize contact of the patient with infectious agents, use...

...prophylactic granulocyte transfusions.

It is not necessary that such cotreatment drugs be included in the **TGF- beta** compositions per se although this will be convenient where such drugs are delivered by the...

...g., antagonists to the activity of **TNF- alpha** or **IL-1**, or the abovedescribed neutralizing **antibodies**.

When employed together with the **TGF-beta**, such agents (other than antibiotics) preferably are employed in lesser dosages than when used alone...

...will contain greater than about 0.5 nmole, generally about 0.05 mu mole, of **TGF-beta**, about 0.0003 to 0.05 mu mole of anti-lipopolysaccharide IgG, and about...

...by reference.

#### EXAMPLE 1

The experiments described below were designed to determine the effects of **TGF- beta** on LPS-induced **TNF- alpha** production in vivo.

Five groups of female BALB/c mice...  
...four hours before time 0 (when LPS was administered) and at time 0.  
Group C: **TGF- beta** at 1 mu g/mouse was administered iv at 100 mu l/mouse at four...

...at time 0) at 5 mu g/mouse iv at 100 mu l/mouse. The **TGF-beta** employed in this experiment was human **TGF- beta 1** obtained recombinantly as described in EP Publ. No. 200,341 published Dec. 10, 1986, the disclosure of which is incorporated herein by reference. The diluent employed for the **TGF- beta** was 20 mM sodium acetate buffer, pH 4.

Three mice per group were sacrificed and...0.2

11	90'	PBS	<0.2	
12		PBS	<0.2	
13		1 mu g <b>TGF- beta</b>	72 +/- 6	
14	60'	1 mu g <b>TGF- beta</b>	409 +/- 19	236 +/- 97
15		1 mu g <b>TGF- beta</b>	227 +/- 14	
16		1 mu g <b>TGF- beta</b>	97 +/- 8	
17	90'	1 mu g <b>TGF- beta</b>	203 +/- 17	241 +/- 97
18		1 mu g <b>TGF- beta</b>	423 +/- 47	
19		5 mu g <b>TGF- beta</b>	443 +/- 30	
20	60'	5 mu g <b>TGF- beta</b>	389 +/- 20	649 +/- 233
21		5 mu g <b>TGF- beta</b>	1114 +/- 72	
22		5 mu g <b>TGF- beta</b>	601 +/- 2	
23	90'	5 mu g <b>TGF- beta</b>	379 +/- 40	561 +/- 324

24 5 mu g TGF- **beta** 705 +/- 68  
 25 10 mu g TGF- **beta** 1039 +/- 64  
 26 60' 10 mu g TGF- **beta** 401 +/- 76 791 +/- 197  
 27 10 mu g TGF- **beta** 935 +/- 67  
 28 10 mu g TGF- **beta** 2292 +/- 24  
 29 90' 10 mu g TGF- **beta** 2118 +/- 83 1842 +/- 365  
 30 10 mu g TGF- **beta** 1118 +/- 13

The results indicate that TGF- **beta** inhibits endotoxin-induced TNF- alpha production at lower doses; at higher doses it significantly enhances...

...alpha was detectable in controls and in mice two hours after 5 mu g of TGF- **beta** was administered ip, 770 U/ml of TNF- alpha were detected two hours after 10...

...g of endotoxin was administered and 240 U/ml of TNF- alpha were detected when TGF- **beta** (5 mu g) was injected simultaneously with 10 mu g of endotoxin.

## EXAMPLE 2

First...

... animals. The mice were permitted food ad libitum. One group received 5 mu g of TGF- **beta** at --24 hours, the second at the time of endotoxin administration, the third at --24...

... treated with phosphate buffered saline at 0.5 ml at --24 hours. The administration of TGF- **beta** and IL-1 was intraperitoneal and the administration of the endotoxin was intravenous at 200 mu g or 0.2 ml per injection.

The TGF- **beta** employed in this experiment was the same as that used in Example 1. After purification, the TGF- **beta** was formulated in acetic acid buffer at pH 5 to 5.4. The IL-1...group of mice versus days is shown in FIG. 3, where the crosses are the TGF- **beta** pretreatment, the closed circles are the TGF- **beta** pretreatment/simultaneous treatment, the closed triangles are the TGF- **beta** simultaneous treatment, the dashed line is the IL-1 treatment, and the asterisks are the TGF- **beta**

5 mu g -24 hr. 30  
 TGF- **beta**  
 5 mu g/5 mu g  
 -24/0 hr. 40

TGF- **beta**  
 5 mu g 0 hr. 50  
 IL-1 2000 units -24 hr. 70

PBS (control Percent mortality was reduced in all cases over the control when TGF- **beta** was employed. The percent mortality was the lowest when the TGF- **beta** was administered once at --24 hours.

## Second Endotoxin Tolerance Model

The above experiment was repeated...

... iv dose of the endotoxin, groups of ten mice, and varying ip doses of the TGF- **beta** used above (i.e., 5 mu g/0.25 ml at 24 hours before endotoxin...

...shown in FIG. 4, where the open squares are the control, the crosses are



the **TGF- beta** 24 hours before endotoxin administration, the open circles are 5 mu g **TGF- beta** simultaneously with endotoxin administration, and the closed circles are 10 mu g **TGF- beta** simultaneously with endotoxin administration. The 10 mu g/mouse dose of **TGF- beta** was found to enhance mortality after endotoxin administration.

#### Septicemia/Cecal Ligation Model

Because of the potential immunosuppressive effects of **TGF- beta** a study was performed to see if **TGF- beta** would increase the susceptibility of mice to bacterial infection due to a puncture wound.

**TGF- beta** and IL-1 were administered separately, intraperitoneally, at 5 mu g dose of **TGF- beta** and at 2000 units dose of IL-1 once at 24 hours prior to surgery...

... circles represent the control, the closed circles represent the IL-1, and the crosses represent **TGF- beta**. IL-1 was found to reduce mortality relative to the control, whereas **TGF- beta** did not decrease or substantially increase mortality relative to the control. Thus, **TGF- beta**, which is a known immunosuppressive agent, did not increase the mortality of the mice.

EXAMPLE the lethal effects of overwhelming bacterial infection. The administration of **antibodies** to TNF- alpha, in contrast, protects animals from the lethal effects of septic shock. As **TGF- beta** can decrease endotoxin-stimulated release of TNF- alpha in vitro and in vivo (J. Exo. Med., 166: 571 (1987)), the ability of **TGF- beta** to inhibit E. coli-induced septic shock was investigated, as described below:

Fischer 344 male rats of 180-200 g were administered intraperitoneally 10 mu g of either recombinant **TGF- beta** 1 (prepared as described in Example 1 in a buffer comprising 20 mM sodium acetate pH 5 with 0.1% weight:volume human serum albumin), recombinant **TGF- beta** 2 (obtained from Dr. Adriano Fontana, Zurich, Switzerland, as a lyophilized pellet - - - see WO 88It was found that 1-25 mu g of **TGF- beta** 1 in this rat model was effective in reducing mortality versus the diluent control., however, a dose of 50 mu g of **TGF- beta** sub 1 increased mortality versus the control. This is consistent with the dose-dependent results...

...about 5 mu g/kg to 1 mg/kg of patient body weight and the **TGF- beta** is administered parenterally on a daily basis.

3. The method of claim 1 wherein the **TGF- beta** is administered to a patient having a microbial infection but not yet showing symptoms of septic shock.

4. The method of claim 3 wherein the **TGF- beta** is administered by intravenous infusion or intraperitoneally.

5. The method of claim 2 wherein the **TGF- beta** is administered intraperitoneally to a patient having a microbial infection but not yet showing symptoms of septic shock.

6. The method of claim 1 wherein the **TGF- beta** is administered with a pharmaceutically effective amount of mafenide acetate, an anti-microbial agent, a...

...of two or more of these substances.

7. The method of claim 1 wherein the **TGF- beta** is human **TGF- beta**.

8. The method of claim 1 wherein the TGF- beta is TGF-  
beta sub 1, TGF- beta sub 2, or TGF- beta sub  
3.

9. The method of claim 7 wherein the TGF- beta is TGF-  
beta sub 1, TGF- beta sub 2, or TGF- beta sub  
3,  
? ds

Set	Items	Description
S1	386	(TGF(W)BETA) AND (TREAT? OR THERAP? OR INHIBIT? OR SUPPRES- S? OR DECREAS?) (30N) (GLOMERULONEPHRITIS OR DIABETES OR ARDS OR RESPIRATORY(W)DISTRESS OR EXTRACELLULAR(W)MATRIX)
S2	86	S1 AND (TGF(W)BETA) (20N) (ANTIBOD?)

2/3/70 (Item 70 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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02598776

Utility

INHIBITION OF TRANSFORMING GROWTH FACTOR BETA ACTIVITY  
[Binding with decorin]

PATENT NO.: 5,583,103

ISSUED: December 10, 1996 (19961210)

INVENTOR(s): Ruoslahti, Erkki I., Rancho Santa Fe, CA (California), US  
(United States of America)  
Yamaguchi, Yu, San Diego, CA (California), US (United States  
of America)

ASSIGNEE(s): La Jolla Cancer Research Foundation, (A U.S. Company or  
Corporation), La Jolla, CA (California), US (United States of  
America)

[Assignee Code(s): 9506]

APPL. NO.: 8-212,311

FILED: March 14, 1994 (19940314)

This application is a continuation of application Ser. No. 08-050,762, filed Apr. 20, 1993, now abandoned, which is a continuation of application Ser. No. 07-467,888, filed on Jan. 22, 1990, now abandoned, which is a continuation of application Ser. No. 07-212,702, filed Jun. 28, 1988 now abandoned.

This invention was made with support of government grants CA 30199, CA 42507 and CA 28896 from the National Cancer Institute. Therefore, the United States government may have rights in the invention.

2/3/61 (Item 61 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02675128

Utility

USE OF FIBROMODULIN TO PREVENT OR REDUCE DERMAL SCARRING

PATENT NO.: 5,654,270  
ISSUED: August 05, 1997 (19970805)  
INVENTOR(s): Ruoslahti, Erkki I., Rancho Santa Fe, CA (California), US  
(United States of America)  
Longaker, Michael T., San Francisco, CA (California), US  
(United States of America)  
Whitby, David J., Adel, GB (United Kingdom)  
ASSIGNEE(s): La Jolla Cancer Research Foundation, (A U.S. Company or  
Corporation), La Jolla, CA (California), US (United States of  
America)  
[Assignee Code(s): 9506]  
EXTRA INFO: Assignment transaction [Reassigned], recorded February 17,  
1998 (19980217)  
APPL. NO.: 8-303,238  
FILED: September 08, 1994 (19940908)

This application is a continuation of application Ser. No. 07-978,931, filed Nov. 17, 1992, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-882,345, filed May 13, 1992, now abandoned, which is a continuation of U.S. Ser. No. 07-792,192, filed Nov. 14, 1991, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-467,888, filed Jan. 22, 1990, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-212,702, filed Jun. 28, 1988, now abandoned.

This invention was made with support of government grants CA 30199, CA 42507 and CA 28896 from the National Cancer Institute. Therefore, the United States government may have rights in the invention.

DECORIN FRAGMENTS INHIBITING CELL REGULATORY FACTORS

PATENT NO.: 5,705,609

ISSUED: January 06, 1998 (19980106)

INVENTOR(s): Ruoslahti, Erkki I., Rancho Santa Fe, CA (California), US  
(United States of America)  
Pierschbacher, Michael D., San Diego, CA (California), US  
(United States of America)  
Cardenas, Jose, San Diego, CA (California), US (United States  
of America)  
Craig, William, San Diego, CA (California), US (United States  
of America)  
Mullen, Daniel G., San Diego, CA (California), US (United  
States of America)

ASSIGNEE(s): La Jolla Cancer Research Foundation, (A U.S. Company or  
Corporation), La Jolla, CA (California), US (United States of  
America)  
[Assignee Code(s): 9506]

APPL. NO.: 8-442,063

FILED: May 16, 1995 (19950516)

This application is a continuation of application Ser. No. 07-865,652, filed Apr. 3, 1992, now abandoned which is a continuation-in-part of application Ser. No. 07-792,192, filed Nov. 14, 1991, now abandoned which is a continuation-in-part of Ser. No. 07-467,888, filed Jan. 22, 1990, now abandoned which is a continuation-in-part of Ser. No. 07-212,702, filed Jun. 28, 1988.

This invention was made with support of government grants CA 30199, CA 42507 and CA 28896 from the National Cancer Institute. Therefore, the

2/3/22 (Item 22 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02881953

Utility

CLONING AND EXPRESSION OF SIMIAN TRANSFORMING GROWTH FACTOR .BETA.1

PATENT NO.: 5,844,085

ISSUED: December 01, 1998 (19981201)

INVENTOR(s): Purchio, Anthony F., Seattle, WA (Washington), US (United States of America)

Gentry, Larry, Maumee, OH (Ohio), US (United States of America)

Twardzik, Daniel, Bainbridge Island, WA (Washington), US (United States of America)

Brunner, Amy M., Seattle, WA (Washington), US (United States of America)

ASSIGNEE(s): Bristol-Myers Squibb Pharmaceutical Research Institute-Seattle, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)

APPL. NO.: 7-958,522

FILED: October 08, 1992 (19921008)

This application is a continuation of U.S. application Serial No. 07-353,728, filed May 17, 1989, now abandoned, which is a continuation-in-part of application Ser. No. 07-350,171, filed Apr. 17, 1989, now abandoned, which is a continuation-in-part of application Ser. No. 07-285,917, filed Dec. 16, 1988, now abandoned, which is a continuation-in-part of application Ser. No. 07-189,894, filed May 3, 1988, now abandoned, which is a continuation-in-part of application Ser. No. 07-147,842, filed Jan. 25, 1988, now abandoned, which is a continuation-in-part of application Ser. No. 07-055,662, filed May 29, 1987, now abandoned, each of which application is incorporated by reference

? ds

Set	Items	Description
S1	386	(TGF(W)BETA) AND (TREAT? OR THERAP? OR INHIBIT? OR SUPPRES- S? OR DECREAS?) (30N) (GLOMERULONEPHRITIS OR DIABETES OR ARDS OR RESPIRATORY (W)DISTRESS OR EXTRACELLULAR (W)MATRIX)
S2	86	S1 AND (TGF(W)BETA) (20N) (ANTIBOD?)

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\$0.19 Estimated cost File1

\$0.01 TYMNET

\$0.20 Estimated cost this search

\$0.20 Estimated total session cost 0.058 DialUnits

File 410:Chronolog(R) 1981-1999 Sep/Oct

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SYSTEM:OS - DIALOG OneSearch
File 5:Biosis Previews(R) 1969-1999/Oct W2
(c) 1999 BIOSIS
File 73:EMBASE 1974-1999/Oct W2
(c) 1999 Elsevier Science B.V.
File 155:MEDLINE(R) 1966-1999/Dec W4
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First update for 2000 will be added in mid-December.
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(c) 1999 Derwent Publ Ltd
*File 357: Derwent changes DialUnit pricing from May 1, 1999. See
HELP DERWENT for details.
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*File 652: Reassignment data now current through 07/09/99
Reexamination, extension, expiration, reinstatement updated weekly.
File 653:US Patents Fulltext 1980-1989
(c) format only 1999 The Dialog Corp.
*File 653: Reassignment data now current through 07/09/99.
Reexamination, extension, expiration, reinstatement updated weekly.
File 654:US Pat.Full. 1990-1999/Oct 26
(c) format only 1999 The Dialog Corp.
*File 654: Reassignment data current through 07/09/99.

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? s tgf(w)beta and antibod? and (extracellular(w)matrix or glomerulonephritis
or respiratory(w)distress or ard or cirrhosis)

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Processing

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      47605 TGF
    1675719 BETA
      34290 TGF(W)BETA
    1556301 ANTIBOD?
    354776 EXTRACELLULAR
    481687 MATRIX
    78612 EXTRACELLULAR(W)MATRIX
    56794 GLOMERULONEPHRITIS
    748012 RESPIRATORY
    93151 DISTRESS
    56715 RESPIRATORY(W)DISTRESS
    3065 ARD
    124987 CIRRHOSIS
S1      1282 TGF(W)BETA AND ANTIBOD? AND (EXTRACELLULAR(W)MATRIX OR
      GLOMERULONEPHRITIS OR RESPIRATORY(W)DISTRESS OR ARD OR
      CIRRHOSIS)

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      1282 S1
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S2 97 S1 AND CIRRHOSIS  
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 S3 95 RD S2 (unique items)  
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 S4 14 S1 AND PY=1988  
 ? rd s4  
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 >>>Records from unsupported files will be retained in the RD set.  
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 ? t s5/7/all

5/7/1 (Item 1 from file: 5)  
 DIALOG(R)File 5:Biosis Previews(R)  
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06605448 BIOSIS NO.: 000087047610  
 TENASCIN COMPLEMENTARY DNA CLONING AND INDUCTION BY **TGF-BETA**

AUTHOR: PEARSON C A; PEARSON D; SHIBAHARA S; HOFSTEENGE J;  
 CHIQUET-EHRISMANN R  
 AUTHOR ADDRESS: FRIEDRICH MIESCHER INST., POSTFACH 2543, CH-4002 BASEL,  
 SWITZ.

JOURNAL: EMBO (EUR MOL BIOL ORGAN) J 7 (10). 1988. 2977-2982.  
 FULL JOURNAL NAME: EMBO (European Molecular Biology Organization) Journal  
 CODEN: EMJOD  
 RECORD TYPE: Abstract  
 LANGUAGE: ENGLISH

ABSTRACT: cDNA clones coding for tenascin, an **extracellular matrix** glycoprotein with a restricted tissue distribution, were isolated from a chicken fibroblast cDNA expression library using a specific tenascin antiserum. **Antibodies** eluted from the cDNA-encoded fusion proteins reacted exclusively with tenascin. Limited trypsin treatment of purified tenascin resulted in a peptide which confirmed the deduced protein sequences. The largest clone encoding 632 amino acids showed a cysteine-rich region containing 13 consecutive epidermal growth factor-like repeats of unusual uniformity. Northern blot analysis revealed 8- to 9-kb messages. Tenascin is shown to be induced in vitro by fetal calf serum as well as by transforming growth factor .beta. (**TGF-.beta.**). A 4-fold increase in tenascin secretion by chick embryo fibroblasts was seen after **TGF-.beta.** treatment. The induction of tenascin protein synthesis was preceded by an increase of tenascin mRNA as determined by Northern blot analysis. The induction of tenascin was compared with fibronectin. The accumulation of the two **extracellular matrix** proteins in the medium was differentially affected by fetal calf serum and **TGF-.beta.** and the increase was in both cases higher for tenascin.

5/7/2 (Item 2 from file: 5)  
 DIALOG(R)File 5:Biosis Previews(R)  
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06563477 BIOSIS NO.: 000087005638

MODULATION OF KERATINOCYTE MOTILITY CORRELATION WITH PRODUCTION OF  
**EXTRACELLULAR MATRIX** MOLECULES IN RESPONSE TO GROWTH  
PROMOTING AND ANTIPROLIFERATIVE FACTORS

AUTHOR: NICKOLOFF B J; MITRA R S; RISER B L; DIXIT V M; VARANI J  
AUTHOR ADDRESS: DEP. PATHOL., UNIV. MICH. MED. SCH., M4232 MED. SCI. I,  
1301 CATHERINE ROAD, ANN ARBOR, MICH. 48109-0602.

JOURNAL: AM J PATHOL 132 (3). 1988. 543-551.  
FULL JOURNAL NAME: American Journal of Pathology  
CODEN: AJPAA  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

ABSTRACT: Normal human epidermal keratinocytes (KC) grown under conditions that maintain the undifferentiated state are highly motile. Migration of these cells as measured in two different assays (migration out of an agarose drop explant, and into micropore filters in a modified Boyden chamber), is stimulated by fibronectin (FN) and to a lesser extent by thrombospondin (TSP). In contrast, laminin (LN) inhibits KC migration. Cultivation of the cells for 1 day under conditions that induce differentiation (ie, in the presence of 1.4 mM Ca2+) suppresses KC motility. A number of soluble growth modulating polypeptide factors also influence KC migration. Transforming growth factor-beta (TGF-.beta.) and epidermal growth factor (EGF) stimulate KC motility. These factors simultaneously induce KC production of FN and a significant portion of the stimulated motility can be inhibited with **antibodies** to FN. EGF and somatomedin-C (SM-C), but not TGF-.beta., also stimulate TSP production while EGF and SM-C (but not TGF-.beta.) induce KC proliferation. In contrast to these factors, interferon-gamma (INF-.gamma.) inhibits KC production of both FN and TSP and concomitantly inhibits both motility and proliferation. These data suggest that KC properties essential for normal wound healing (ie, motility and proliferation) are regulated by both **extracellular matrix** molecules and soluble peptide factors. Finally, these effects of various growth promoting and antiproliferative factors on KCs may, in part, be mediated through alteration in the endogenous production of **extracellular matrix** molecules by KCs.

5/7/3 (Item 3 from file: 5)  
DIALOG(R)File 5: Biosis Previews(R)  
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06156333 BIOSIS NO.: 000085119485

TRANSFORMING GROWTH FACTOR BETA STIMULATES THE EXPRESSION OF FIBRONECTIN  
AND OF BOTH SUBUNITS OF THE HUMAN FIBRONECTIN RECEPTOR BY CULTURED HUMAN  
LUNG FIBROBLASTS

AUTHOR: ROBERTS C J; BIRKENMEIER T M; MCQUILLAN J J; AKIYAMA S K; YAMADA S  
S; CHEN W-T; YAMADA K M; MCDOLAND J A  
AUTHOR ADDRESS: BOX 8052, WASH. UNIV. SCH. MED., 660 S. EUCLID, ST. LOUIS,  
MO. 63110.

JOURNAL: J BIOL CHEM 263 (10). 1988. 4586-4592.  
FULL JOURNAL NAME: Journal of Biological Chemistry  
CODEN: JBCHA  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

ABSTRACT: Transforming growth factors of the .beta.-class (TGFs-.beta.) stimulate **extracellular matrix** synthesis and have been implicated in embryogenesis, wound healing, and fibroproliferative responses to tissue injury. Because cells communicate with several

**extracellular matrix** components via specific cell membrane receptors, we hypothesized that TGFs-.beta. may also regulate the expression of such receptors. We confirmed that **TGF-.beta.1** increases the expression of fibronectin, an adhesive glycoprotein expression during embryogenesis and tissue remodeling. Based upon the 48-72 h period required for a maximal fibroproliferative response to dermal injection of **TGF-.beta.1**, we exposed human fetal lung fibroblasts (IMR-90) to **TGF-.beta.1** for periods up to 48 h in vitro. We observed as much as 6-fold increases in fibronectin synthesis by 24 h as previously reported for fibroblastic cells (Igotz, R. A., and Massague, J. (1986) J. Biol. Chem. 261, 4337-4345; Igotz, R. A., Endo, T., and Massague, J. (1987) J. Biol. Chem. 262, 6443-6446; Raghov, R., Postlethwaite, A. E., KeskiOja, J., Moses, H. L., and Kang, A. H. (1987) J. Clin. Invest. 79, 1285-1288), but up to 30-fold increases by 48 h. These increases are accompanied by similar increases in fibronectin mRNA levels which are prevented by actinomycin D treatment. Using a monospecific **antibody** raised to the human placental fibronectin receptor complex, we found that **TGF-.beta.1** stimulated fibronectin receptor synthesis up to 20-40-fold and increase mRNA levels encoding both the .alpha. and .beta.-subunits up to 3-fold, compared to control IMR-90 in serum-free medium. Actinomycin D blocks **TGF-.beta.1**-mediated increases in receptor mRNA levels. The earliest detectable **TGF-.beta.**-mediated increases in fibronectin receptor complex protein synthesis and mRNA levels occur at 8 h, whereas the earliest increases in fibronectin protein synthesis and mRNA levels occur at 12 h. These results demonstrate that **TGF-.beta.1** stimulates fibronectin receptor synthesis, extending the diverse stimulatory activities of this polypeptide to matrix receptors. In addition, because fibronectin matrix assembly may involve the fibronectin cell adhesive receptor complex, increased receptor expression may help drive deposition into matrix.

5/7/4 (Item 1 from file: 155)  
 DIALOG(R)File 155:MEDLINE(R)  
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06005315 89034456  
 Altered structure of the hybrid cell surface proteoglycan of mammary epithelial cells in response to transforming growth factor-beta.  
 Rasmussen S; Rapraeger A  
 Department of Pathology, University of Wisconsin, Madison 53706.  
 J Cell Biol (UNITED STATES) Nov 1988, 107 (5) p1959-67, ISSN 0021-9525 Journal Code: HMV  
 Contract/Grant No.: HD-21881, HD, NICHD  
 Languages: ENGLISH  
 Document type: JOURNAL ARTICLE  
 Transforming growth factor beta (**TGF-beta**) is a polypeptide growth factor that affects the accumulation of **extracellular matrix** by many cell types. We have examined the ability of mouse mammary epithelial (NMuMG) cells to respond to **TGF-beta** and assessed the effect of the growth factor on the expression of their cell surface heparan sulfate/chondroitin sulfate hybrid proteoglycan. NMuMG cells respond maximally to 3 ng/ml **TGF-beta** and the response is consistent with occupancy of the type III receptor. However, cells that are polarized, as shown by sequestration of the cell surface PG at their basolateral surfaces, must have the growth factor supplied to that site for maximal response. Immunological quantification of proteoglycan core protein on treated cells suggests that the cells have an unchanging number of this proteoglycan at their cell surface. Nonetheless, metabolic labeling with radiolabeled shows a approximately 2.5-fold increase in 35S04-glycosaminoglycans in this proteoglycan fraction, defined either by its lipophilic, antigenic, or cell surface properties. Kinetic studies indicate that the enhanced radiolabeling is due to augmented synthesis, rather than slower degradation. Analysis of the glycosaminoglycan composition of the proteoglycan shows an increased amount of chondroitin

sulfate, suggesting that the increased labeling per cell may be attributed to an augmented synthesis of chondroitin sulfate glycosaminoglycan on the core protein that also bears heparan sulfate, thus altering the proportions of these two glycosaminoglycans on this hybrid proteoglycan. We conclude that TGF-beta may affect NMuMG cell behavior by altering the structure and thus the activity of this proteoglycan.

5/7/5 (Item 2 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
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05946952 89276133

Palate development.

Ferguson MW

Department of Cell & Structural Biology, University of Manchester, UK.

Development (ENGLAND) 1988, 103 Suppl p41-60, ISSN 0950-1991

Journal Code: ECW

Languages: ENGLISH

Document type: JOURNAL ARTICLE; REVIEW; REVIEW, ACADEMIC

In all vertebrates, the secondary palate arises as bilateral outgrowths from the maxillary processes. In birds and most reptiles, these palatal shelves grow initially horizontally, but do not fuse with each other resulting in physiological cleft palate. In crocodilians, shelf fusion occurs resulting in an intact secondary palate. Mammalian palatal shelves initially grow vertically down the side of the tongue, but elevate at a precise time to a horizontal position above the dorsum of the tongue and fuse with each other to form an intact palate. Palatal shelf-elevation is the result of an intrinsic shelf elevating force, chiefly generated by the progressive accumulation and hydration of hyaluronic acid. In all vertebrates the nasal epithelium differentiates into pseudostratified ciliated columnar cells and the oral epithelia differentiates into stratified squamous cells, but the medial edge epithelial (MEE) phenotype differs in different groups. In mammals, the MEE of opposing shelves adhere to each other to form an epithelial seam which then disrupts by cell death and cell migration into the mesenchyme accompanied by an epitheliomesenchymal transformation. In birds, the MEE keratinize resulting in cleft palate whereas, in alligators, the MEE migrate onto the nasal aspect of the palate. In all vertebrates, this regional, temporal and species-specific epithelial differentiation is specified by the underlying mesenchyme. Signalling of this interaction is complex but involves both **extracellular matrix** and soluble factors e.g. minor collagen types, tenascin, EGF, TGF alpha, TGF beta, PDGF, FGF. These soluble growth factors have a biphasic effect: directly on the epithelia and on the mesenchyme where they stimulate or inhibit cell division and synthesis of specific **extracellular matrix** molecules. The **extracellular matrix** molecules (and bound growth factors) synthesized by the mesenchymal cells may then directly affect the epithelium. These signals cause differential gene expression via second messenger systems e.g. cAMP, cGMP, Ca<sup>2+</sup>, pH, pI etc. Molecular markers for nasal, medial and oral epithelial cell differentiation include the types of cytokeratin intermediate filaments and specific cell surface molecules recognized by monoclonal **antibodies**: the genes for such molecules are probably expressed in response to mesenchymal signals. Using such an approach, it is possible to go from a morphological description of palate development to a cellular analysis of the mechanisms involved and then to identification of candidate genes that may be important for screening and diagnosis of cleft palate. (101 Refs.)

5/7/6 (Item 3 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
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05928871 89039850

Transforming growth factor beta increases cell surface binding and

assembly of exogenous (plasma) fibronectin by normal human fibroblasts.

Allen-Hoffmann BL; Crankshaw CL; Mosher DF

Department of Pathology, University of Wisconsin, Madison 53706.

Mol Cell Biol (UNITED STATES) Oct 1988, 8 (10) p4234-42, ISSN 0270-7306 Journal Code: NGY

Contract/Grant No.: HL 21644, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Transforming growth factor beta (**TGF-beta**) enhances the cell surface binding of 125I-fibronectin by cultured human fibroblasts. The effect of **TGF-beta** on cell surface binding was maximal after 2 h of exposure to TGF-beta and did not require epidermal growth factor or protein synthesis. The enhancement was dose dependent and was found with the 125I-labeled 70-kilodalton amino-terminal fragment of fibronectin as well as with 125I-fibronectin. Treatment of cultures with **TGF-beta** for 6 h resulted in a threefold increase in the estimated number of fibronectin binding sites. The increase in number of binding sites was accompanied by an increased accumulation of labeled fibronectin in detergent-insoluble **extracellular matrix**. The effect of **TGF-beta** was biphasic; after 6 h of exposure, less labeled fibronectin bound to treated cultures than to control cultures. Exposure of cells to **TGF-beta** for greater than 6 h caused a two- to threefold increase in the accumulation of cellular fibronectin in culture medium as detected by a quantitative enzyme-linked immunosorbent assay. The second phase of the biphasic effect and the increase in soluble cellular fibronectin were blocked by cycloheximide. Immunofluorescence staining of fibroblast cultures with antifibronectin revealed that **TGF-beta** caused a striking increase in fibronectin fibrils. The 70-kilodalton amino-terminal fragment of fibronectin, which blocks incorporation of fibronectin into **extracellular matrix**, blocked anchorage-independent growth of NRK-49F cells in the presence of epidermal growth factor. Our results show that an increase in the binding and rate of assembly of exogenous fibronectin is an early event preceding the increase in expression of **extracellular matrix** proteins. (ABSTRACT TRUNCATED AT 250 WORDS)

5/7/77 (Item 4 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

(c) format only 1999 Dialog Corporation. All rts. reserv.

05912931 88253282

Induction of carcinoembryonic antigen secretion and modulation of protein secretion/expression and fibronectin/laminin expression in human colon carcinoma cells by transforming growth factor-beta.

Chakrabarty S; Tobon A; Varani J; Brattain MG

Bristol-Baylor Laboratory, Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030.

Cancer Res (UNITED STATES) Jul 15 1988, 48 (14) p4059-64, ISSN

0008-5472 Journal Code: CNF

Contract/Grant No.: CA34432, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We have recently reported that **TGF-beta** induces a response similar to that of planar polar differentiation promoters in human colon carcinoma MOSER cells. N,N-Dimethylformamide and **TGF-beta** had similar effects on MOSER cells with respect to reversible inhibition of growth (both in monolayer culture and semisolid medium), induction of fibronectin expression and the induction of morphological alterations (Cancer Res., 47:2950-2954, 1987). Since the expression of carcinoembryonic antigen (CEA) has been reported to be modulated by planar polar compounds that promote differentiation in colon carcinomas, we addressed the issue of whether the differentiation-like effects of **TGF-beta** on these cells would also encompass modulation of CEA expression in the MOSER cells. The biological modulating effects of **TGF-beta** on **extracellular matrix** glycoprotein expression and the expression

and secretion of cellular proteins were also studied in view of the reported modulating effects of this growth factor on untransformed, noncolonic cells. In this communication we report that **TGF-beta** induced the synthesis of fibronectin and laminin but not collagen IV. **TGF-beta** also induced CEA secretion in a dose-dependent manner. Elevated CEA secretion was detected following 48 h of **TGF-beta** treatment and a 16-fold increase in CEA secretion was observed following 7 days of treatment. The cells were committed to secrete CEA following one dose of **TGF-beta** treatment. The enhanced expression of four cellular proteins (Mr 42,000, Mr 48,000, Mr 52,000, and Mr 55,000) and the enhanced secretion of three proteins (Mr 66,000, Mr 200,000, and Mr 400,000) were also induced. Some of these protein alterations were detected as early as 6-24 h following **TGF-beta** treatment. It is concluded that **TGF-beta** modulated the production and secretion of CEA, the synthesis of fibronectin and laminin, and the expression and secretion of several cellular proteins in the colon carcinoma MOSER cells. To our knowledge, this is the first report on the modulation of CEA and laminin by **TGF-beta** in tissue-cultured cells, and is the first report on the modulation of cellular proteins by this growth factor in human colon carcinoma cells.

5/7/8 (Item 5 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
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05500564 88198381

Phenotypic modulation of endothelial cells by transforming growth factor-beta depends upon the composition and organization of the **extracellular matrix**.

Madri JA; Pratt BM; Tucker AM  
Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06510.

J Cell Biol (UNITED STATES) Apr 1988, 106 (4) p1375-84, ISSN 0021-9525 Journal Code: HMV

Contract/Grant No.: HL-27383, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Transforming growth factor beta (**TGF-beta**) is angiogenic in vivo. In vitro, endothelial cell proliferation is inhibited by **TGF-beta**. We have correlated this inhibitory effect with an increase in cellular fibronectin synthesis and deposition in a two-dimensional culture system using specific matrix coatings. The inhibitory effect was mimicked by addition of soluble fibronectin to cultures. In contrast, **TGF-beta** was found to elicit the formation of tube-like structures (mimicking angiogenesis) when microvascular endothelial cells were grown in three-dimensional collagen gels. In this culture system **TGF-beta** elicited rapid extensive formation of complex, branching, tube-like structures, while cell proliferation was not inhibited. These data confirm and support the hypothesis that **TGF-beta** is angiogenic and may exert some of its effects through modulation of matrix synthesis and are consistent with the hypothesis that the organization of the extracellular environment influences cellular responses to this "panregulin."

5/7/9 (Item 1 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02723297

Reissue  
POLYPEPTIDE CARTILAGE-INDUCING FACTORS FOUND IN BONE  
[Mixture of transforming growth factor, carriers and polypeptide]

PATENT NO.: RE35,694

ISSUED: December 16, 1997 (19971216)  
INVENTOR(s): Seyedin, Saeid, Saratoga, CA (California), US (United States of America)  
Thomas, Thomas, West Roxbury, MA (Massachusetts), US (United States of America)  
Bentz, Hanne, Newark, CA (California), US (United States of America)  
Ellingsworth, Larry, San Jose, CA (California), US (United States of America)  
Armstrong, Rosa, Palo Alto, CA (California), US (United States of America)  
ASSIGNEE(s): Celtrix Pharmaceuticals, Inc , (A U.S. Company or Corporation)  
, Santa Clara, CA (California), US (United States of America)  
[Assignee Code(s): 28382]  
APPL. NO.: 8-257,472  
FILED: June 09, 1994 (19940609)

Reissue (first reissue) of patent no.: 4,843,063, issued: June 27, 1989 (19890627), serial no.: 7-204,173, filed: June 08, 1988 (19880608)

#### CROSS-REFERENCE TO RELATED APPLICATION

This application is a (*italic start*) continuation of application Ser. No. 07-664,766, filed Mar. 5, 1991 abandoned which is a RE of 07-204,173, filed Jun. 8, 1988, now U.S. Pat. No. 4,843,063, which is a (*italic end*) continuation of copending U.S. Ser. No. 129,864, filed Dec. 10, 1987, now U.S. Pat. No. 4,774,322, issued Sept. 27, 1988, which is a continuation of U.S. Ser. No. 767,144, filed Aug. 19, 1985, now abandoned, which is a continuation-in-part of U.S. Ser. No. 630,938, filed July 16, 1984, now abandoned.

FULL TEXT: 796 lines

#### ABSTRACT

Two proteins that are found in bone and that have in vivo chondrogenic/osteogenic activity in combination with a co-factor are described. Both proteins also were active in combination with EGF in the in vitro **TGF- beta** assay. Each has a molecular weight of approximately 26,000 daltons by SDS-PAGE. Each is reduced to a single polypeptide indicating that the proteins are probably homodimers. One has an N-terminal sequence identical to that of human placenta-derived **TGF- beta** whereas the other has an N-terminal sequence that is different from that of **TGF- beta** derived from human placenta. The two proteins may be purified to homogeneity using RP-HPLC or acetic acid-urea gel electrophoresis.

We claim: [ 1. A method of promoting proliferation of cells in an animal comprising

administering to the animal a composition comprising a **TGF-beta** activating agent, a pharmaceutically acceptable carrier, and a polypeptide that (a) is found in mammalian bone, (b) is active in the **TGF-beta** assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide and activating agent are present in amounts sufficient to promote bone growth. ] [ 2. The method of claim 1 wherein each chain of said dimer has the following N-terminal sequence: Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp-. ] [ 3. A method for inducing cartilage and bone formation for repairing, replacing, and or augmenting cartilage and bone tissue in animals, which method comprises:

administering to a mammal in need of bone repair, replacement, or augmentation a composition comprising a **TGF-beta** activating agent, a pharmaceutically acceptable excipient, and a polypeptide the (a) is found in mammalian bone, (b) is active in the **TGF-beta**



assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide and activating agent are present in amounts sufficient to promote bone growth. ] [ 4. The method of claim 3 wherein each chain of said dimer has the following N-terminal sequence: Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-

Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp-. ] [ 5. A method for treating osteoporosis or osteopetrosis, which comprises:

systemically administering an effective amount of a composition comprising a **TGF-beta** activating agent, a pharmaceutically acceptable excipient, and a polypeptide that (a) is found in mammalian bone, (b) is active in the **TGF-beta** assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide and activating agent are present in amounts sufficient to promote bone growth. ] [ 6. The method of claim 5 wherein each chain of said dimer has the following N-terminal sequence: Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp-. ] [ 7. The method of claim 3 wherein each chain of said dimer has the following N-terminal sequence: Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pro-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp. ] [ 8. The method of claim 5 wherein each chain of said dimer has the following N-terminal sequence: Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pr

o-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp. ] (italic start) 9. A method for inducing cartilage and bone formation for repairing, replacing, and/or augmenting cartilage and bone tissue in animals, which method comprises administering to an animal in need of bone repair, replacement, or augmentation a composition comprising a **TGF-beta** activating agent, a pharmaceutically acceptable excipient, and a polypeptide that (a) is found in mammalian bone, (b) is active in the **TGF-beta** assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide and activating agent are present in amounts sufficient to promote bone growth. (italic end) (italic start) 10. The method of claim 9 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp (**TGF-beta** 1). (italic end) (italic start) 11. The method of claim 9 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pr o-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp

(**TGF beta** -2). (italic end) (italic start) . A method for treating osteoporosis or osteopetrosis, which comprises systemically administering to an individual in need of such treatment an effective amount of a composition comprising a **TGF-beta** activating agent, a pharmaceutically acceptable excipient, and a polypeptide that (a) is found in mammalian bone, (b) is active in the **TGF-beta** assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide and activating agent are present in amounts sufficient to promote bone growth. (italic end) (italic start) 13. The method of claim 12 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp (**TGF beta** -1).

(italic end) (italic start) 14. The method of claim 12 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pr o-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp (**TGF beta** -2).

(italic end) (italic start) 15. A method for inducing cartilage and bone formation for repairing, replacing, and/or augmenting cartilage and bone tissue in animals, which method comprises administering to an animal in need of bone repair, replacement, or augmentation a composition comprising a pharmaceutically acceptable excipient and a polypeptide that (a) is found in mammalian bone, (b) is active in the **TGF- beta** assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide is

present in an amount sufficient to promote bone growth. (italic end) (italic start) 16. The method of claim 15 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp (**TGF beta -1**).

(italic end) (italic start) 17. The method of claim 15 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pro-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp (**TGF beta -2**).

(italic end) (italic start) 18. A method for treating osteoporosis or osteopetrosis, which comprises systemically administering to an individual in need of such treatment an effective amount of a composition comprising a pharmaceutically acceptable excipient and a polypeptide that (a) is found in mammalian bone, (b) is active in the **TGF- beta** assay, (c) is

a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide is present in an amount sufficient to promote bone growth. (italic end)

(italic start) 19. The method of claim 18 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp

(**TGF beta -1**). (italic end). I add slashed zero The method of claim 18 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pro-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp (**TGF beta -2**).

(italic end)

? ds

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Set      Items  Description
S1       1282   TGF(W)BETA AND ANTIBOD? AND (EXTRACELLULAR(W)MATRIX OR GLO-
              MERULONEPHRITIS OR RESPIRATORY(W)DISTRESS OR ARD OR CIRRHOSIS)
S2       97     S1 AND CIRRHOSIS
S3       95     RD S2 (unique items)
S4       14     S1 AND PY=1989
S5       9      RD S4 (unique items)
? s s1 and py=1989
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      1282 S1
      1809823 PY=1989
S6      18     S1 AND PY=1989
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? rd s6

>>>Duplicate detection is not supported for File 652.

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>>>Duplicate detection is not supported for File 654.

>>>Records from unsupported files will be retained in the RD set.

...completed examining records

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      S7      12 RD S6 (unique items)
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7/7/1 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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06773252 BIOSIS NO.: 000088082685

EPITHELIAL-MESENCHYMAL CELL TRANSFORMATION IN THE EMBRYONIC HEART CAN BE  
MEDIATED IN PART BY TRANSFORMING GROWTH FACTOR BETA

AUTHOR: POTTS J D; RUNYAN R B

AUTHOR ADDRESS: DEP. ANATOMY, UNIV. IOWA, IOWA CITY, IOWA 52242.

JOURNAL: DEV BIOL 134 (2). 1989. 392-401.

FULL JOURNAL NAME: Developmental Biology

CODEN: DEBIA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Progenitor cells of the valves and membranous septa of the vertebrate heart are formed by transformation of a specific population of endothelial cells into mesenchyme. Previous studies have shown that this epithelial-mesenchymal cell transformation is mediated by a signal produced by the myocardium of the atrioventricular (AV) canal and transferred across the **extracellular matrix**. Data are presented here that transforming growth factor .beta. (**TGF.beta.1** or **TGF.beta.2**), in combination with an explant of ventricular myocardium, will produce an epithelial-mesenchymal transformation by cultured AV canal endothelial cells in vitro. Alone, neither component is capable of producing this effect. The factor provided by the ventricular explant cannot be substituted by either epidermal growth factor or basic fibroblast growth factor. Further experiments show that an **antibody** that blocks **TGF.beta.** activity is effective in preventing the epithelial-mesenchymal cell transformation normally produced by AV canal myocardium. Control **antibodies** are without effect. By immunological criteria, a member of the **TGF.beta.** family of molecules can be demonstrated in the chicken embryo and heart at the time overt valvular formation begins. Together, these data show that **TGF.beta.1** can produce mesenchymal cell formation in vitro and provide evidence that a member of the **TGF.beta.** family is present and plays a role in the process of epithelial-mesenchymal cell transformation in the embryonic heart.

7/7/2 (Item 2 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

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06749421 BIOSIS NO.: 000088058852

MODULATION OF ACTIN MESSENGER RNA IN CULTURED VASCULAR CELLS BY MATRIX  
COMPONENTS AND **TGF-BETA**

AUTHOR: KOCHER O; MADRI J A

AUTHOR ADDRESS: DEP. PATHOL., YALE UNIV. SCH. MED., 310 CEDAR STREET, NEW  
HAVEN, CONN. 06510.

JOURNAL: IN VITRO CELL DEV BIOL 25 (5). 1989. 424-434.

FULL JOURNAL NAME: In Vitro Cellular & Developmental Biology

CODEN: ICDBE

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Alpha-smooth muscle actin is currently considered a marker of smooth muscle cell differentiation. However, during various physiologic and pathologic conditions, it can be expressed, sometimes only transiently, in a variety of other cell types, such as cardiac and skeletal muscle cells, as well as in nonmuscle cells. In this report, the expression of actin mRNAs in cultured rat capillary endothelial cells (RFCs) and aortic smooth muscle cells (SMCs) has been studied by Northern hybridization in two-dimensional cultures seeded on individual **extracellular matrix** proteins and a three-dimensional type I

collagen gels. In two-dimensional cultures, in addition to cytoplasmic actin mRNAs which are normally found in endothelial cell populations, RFCs expressed .alpha.-smooth muscle (SM) actin mRNA at low levels .alpha.-SM actin mRNA expression is dramatically enhanced by TGF-.beta.1. In addition, double immunofluorescence staining with anti-vWF and anti-.alpha.-SM-1 (a monoclonal **antibody** to .alpha.-SM actin) shows that RFCs co-express the two proteins. In three dimensional cultures, RFCs still expressed vWF, but lost staining for .alpha.-SM actin, whereas .alpha.-SM actin mRNA became barely detectable. In contrast to two-dimensional cultures, the addition of TGF-.beta.1 to the culture media did not enhance .alpha.-SM actin mRNA in three-dimensional cultures, whereas it induced rapid capillary tube formation. Actin mRNA expression was modulated in SMCs by **extracellular matrix** components and TGF-.beta.1 with a pattern very different from that of RFCs. Namely, the comparison of RFCs with other cell types such as bovine aortic endothelial cells shows that co-expressions of endothelial and smooth muscle cell markers in very unique to RFCs and occurs only in particular culture conditions. This could be related to the capacity of these microvascular endothelial cells to modulate their phenotype in physiologic and pathologic conditions, particularly during angiogenesis, and could reflect different embryologic origins for endothelial cell populations.

7/7/3 (Item 3 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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06661042 BIOSIS NO.: 000087103219  
EXPRESSION OF TRANSFORMING GROWTH FACTOR-BETA-1 IN SPECIFIC CELLS AND  
TISSUES OF ADULT AND NEONATAL MICE

AUTHOR: THOMPSON N L; FLANDERS K C; SMITH J M; ELLINGSWORTH L R; ROBERTS A  
B; SPORN M B  
AUTHOR ADDRESS: LAB. CHEMOPREVENTION, NATL. CANCER INST., BUILD. 41,  
BETHESDA, MD. 20892.

JOURNAL: J CELL BIOL 108 (2). 1989. 661-670.  
FULL JOURNAL NAME: Journal of Cell Biology  
CODEN: JCLBA  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

ABSTRACT: We have used immunohistochemical techniques to detect transforming growth factor-.beta.1 (TGF-1) in many tissues of adult and neonatal mice. Each of two **antibodies** raised to the amino-terminal 30 amino acids of TGF-.beta.1 selectively stained this molecule in either intracellular or extracellular locations. Strong intracellular staining was found in adrenal cortex, megakaryocytes and other cells of the bone marrow, cardiac myocytes, chondrocytes, renal distal tubules, ovarian glandular cells, and chorionic cells of the placenta. Marked staining of **extracellular matrix** was found in cartilage, heart, pancreas, placenta, skin, and uterus. Staining was often particularly intense in specialized cells of a given tissue, suggesting unique roles for TGF-.beta. within that tissue. Levels of expression of mRNA for TGF-.beta.1 and its histochemical staining did not necessarily correlate in a given tissue, as in the spleen. The present data lend further support to the concept that TGF-.beta. has an important role in controlling interactions between epithelia and surrounding mesenchyme.

7/7/4 (Item 4 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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06658163 BIOSIS NO.: 000087100340

THROMBOSPONDIN IS AN OSTEOBLAST-DERIVED COMPONENT OF MINERALIZED  
EXTRACELLULAR MATRIX

AUTHOR: ROBEY P G; YOUNG M F; FISHER L W; MCCLAIN T D

AUTHOR ADDRESS: BONE RES. BRANCH, NATL. INST. DENTAL RES., NATL. INST.  
HEALTH, BETHESDA, MD. 20892.

JOURNAL: J CELL BIOL 108 (2). 1989. 719-728.

FULL JOURNAL NAME: Journal of Cell Biology

CODEN: JCLBA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Thrombospondin, the most abundant protein of platelet .alpha. granules, is a biosynthetic product of a variety of connective tissue cells and a component of many extracellular matrices. In this study, thrombospondin distribution in bone was investigated using a monoclonal **antibody** specific for the human protein. Thrombospondin was localized in osteoid of undemineralized, frozen sections of fetal subperiosteal bone, and identified as a component of mineralized bone matrix of neonatal and/or young (growing) bone of many animal species by Western blot analysis. Adult human bone cells were demonstrated to contain mRNA for thrombospondin by hybridization of a cDNA thrombospondin probe to a 6.1 kb mRNA. Pulse-chase experiments indicated that the protein was synthesized and the majority was secreted from osteoblastic cells. Treatment of the cells with **TGF-.beta.** (0.01-10 ng/ml) slightly decreased total thrombospondin synthesis, but caused an increase in the retention on newly synthesized thrombospondin in the cell layer/matrix fraction. In cell attachment assays, thrombospondin mediated adhesion, but not spreading of adult human bone cells.

7/7/5 (Item 5 from file: 5)

DIALOG(R)File 5: Biosis Previews(R)

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06637599 BIOSIS NO.: 000087079766

IMMUNODETECTION AND QUANTITATION OF THE TWO FORMS OF TRANSFORMING GROWTH  
FACTOR-BETA **TGF-BETA-1** AND **TGF-BETA-2** SECRETION  
BY CELLS IN CULTURE

AUTHOR: DANIELPOUR D; DART L L; FLANDERS K C; ROBERTS A B; SPORN M B

AUTHOR ADDRESS: LAB. CHEMOPREVENTION, NATL. CANCER INST., BETHESDA, MD.  
20892.

JOURNAL: J CELL PHYSIOL 138 (1). 1989. 79-86.

FULL JOURNAL NAME: Journal of Cellular Physiology

CODEN: JCLLA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: Transforming growth factor beta (**TGF-.beta.**), a potent modulator of cell growth, differentiation, and the expression of **extracellular matrix** components in a variety of cell types, exists as two distinct homodimers (**TGF-.beta.1** and **RGF-.beta.2**), sharing 71% sequence homology. Radioreceptor and previously described radioimmunological assays using rabbit **antibodies** have not been able to distinguish between these two forms. We have developed antisera in turkeys against native **TGF-.beta.1** and **TGF-.beta.2**, each of which specifically blocks both the receptor binding and biological activity of each of these peptides. With these immunological reagents we describe sensitive and specific immunological assays for **TGF-.beta.1** and **TGF-.beta.2** in complex biological fluids. Using these assays we show that both **TGF-.beta.1** and **TGF-.beta.2** are secreted by a variety of

cultured cells, but that some cells secrete predominantly either **TGF-.beta.1** or **TGF-.beta.2** while others secrete both peptides in nearly equal amounts. Our results demonstrate that the expression of each of the two forms of **TGF-.beta.** is independently regulated.

7/7/6 (Item 1 from file: 73)  
DIALOG(R)File 73:EMBASE  
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03896913 EMBASE No: 1989065869

Thrombospondin is an osteoblast-derived component of mineralized **extracellular matrix**

Gehron Robey P.; Young M.F.; Fisher L.W.; McClain T.D.  
Surgery Branch, National Cancer Institute, National Institutes of Health,  
Bethesda, MD 20892 United States  
Journal of Cell Biology ( J. CELL BIOL. ) (United States) 1989, 108/2  
(719-727)

CODEN: JCLBA ISSN: 0021-9525  
DOCUMENT TYPE: Journal  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Thrombospondin, the most abundant protein of platelet alpha granules, is a biosynthetic product of a variety of connective tissue cells and a component of many extracellular matrices. In this study, thrombospondin distribution in bone was investigated using a monoclonal **antibody** specific for the human protein. Thrombospondin was localized in osteoid of undemineralized, frozen sections of fetal subperiosteal bone, and identified as a component of mineralized bone matrix of neonatal and/or young (growing) bone of many animal species by Western blot analysis. Adult human bone cells were demonstrated to contain mRNA for thrombospondin by hybridization of a cDNA thrombospondin probe to a 6.1 kb mRNA. Pulse-chase experiments indicated that the protein was synthesized and the majority was secreted from osteoblastic cells. Treatment of the cells with **TGF-beta** (0.01-10 ng/ml) slightly decreased total thrombospondin synthesis, but caused an increase in the retention on newly synthesized thrombospondin in the cell layer/matrix fraction. In cell attachment assays, thrombospondin mediated adhesion, but not spreading of adult human bone cells.

7/7/7 (Item 1 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
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05889862 89139582

Transforming growth factor-beta 1: histochemical localization with **antibodies** to different epitopes.

Flanders KC; Thompson NL; Cissel DS; Van Obberghen-Schilling E; Baker CC; Kass ME; Ellingsworth LR; Roberts AB; Sporn MB  
Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20892.

J Cell Biol (UNITED STATES) Feb 1989, 108 (2) p653-60, ISSN 0021-9525 Journal Code: HMV

Languages: ENGLISH  
Document type: JOURNAL ARTICLE

We have localized transforming growth factor-beta (**TGF-beta**) in many cells and tissues with immunohistochemical methods, using two polyclonal antisera raised to different synthetic preparations of a peptide corresponding to the amino-terminal 30 amino acids of **TGF-beta**

1. These two **antibodies** give distinct staining patterns; the staining by anti-CC(1-30) is intracellular. This differential staining pattern is consistently observed in several systems, including cultured tumor cells; mouse embryonic, neonatal, and adult tissues; bovine

fibropapillomas; and human colon carcinomas. The extracellular staining by anti-CC(1-30) partially resembles that seen with an antibody to fibronectin, suggesting that extracellular TGF-beta may be bound to matrix proteins. The intracellular staining by anti-LC(1-30) is similar to that seen with two other antibodies raised to peptides corresponding to either amino acids 266-278 of the TGF-beta 1 precursor sequence or to amino acids 50-75 of mature TGF-beta 1, suggesting that anti-LC(1-30) stains sites of TGF-beta synthesis. Results from RIA and ELISAs indicate that anti-LC(1-30) and anti-CC(1-30) recognize different epitopes of this peptide and of TGF-beta 1 itself.

7/7/8 (Item 2 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
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05763468 89194355

Interleukin-4 induces a substance in bone marrow stromal cells that reversibly inhibits factor-dependent and factor-independent cell proliferation.

Peschel C; Green I; Paul WE  
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

Blood (UNITED STATES) Apr 1989, 73 (5) p1130-41, ISSN 0006-4971  
Journal Code: A8G

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Bone marrow-derived stromal cell monolayers pretreated with recombinant interleukin-4 (IL-4) inhibit the growth of hematopoietic cells. This was demonstrated by inhibition of fresh bone marrow-derived, IL-3-induced soft agar colonies as well as by inhibition of proliferation of IL-3-dependent cell lines and of a Friend virus-transformed erythroleukemic cell line. Pretreatment of stromal cells with IL-4 for five to seven days induced the inhibitory activity. IL-4 could then be removed before "plating" the bone marrow cells in soft agar, indicating that the inhibitory activity did not depend on the action of IL-4 on the precursors of the soft agar colonies. The inhibitory activity appears to be mediated by a soluble factor since inhibition was achieved even if the stromal cell layer was separated from the colony forming cells by an "empty" agar layer. However, supernatants of IL-4-induced stromal cell layers had no detectable inhibitory activity. The inhibitory action of the IL-4-pretreated stromal cell lines was not the result of killing of the precursor cells since it could be reversed if the agar layer containing the colony-forming cells was removed from the stromal cell layer and cultured with IL-3. Hydrocortisone (HC) blocked the inhibitory effect if added either in the IL-4 preincubation phase or during the colony formation stage, implying that HC blocked both induction of the inhibitory activity and its release or its effector function. A homogenous long-term stromal cell line could not be induced to exert the inhibitory activity; partial inhibition could be achieved with pure macrophages stimulated with IL-4 and CSF-1, suggesting that the inhibitory activity induced by IL-4 in mixed stromal cell layers may depend on a complex mechanism involving more than one cell type. Northern analysis of RNA from IL-4-induced and uninduced stromal cells indicated that IL-4 did not upregulate expression of CSF-1 or transforming growth factor-beta (TGF-beta) and only modestly increased expression of tumor necrosis factor, suggesting that these cytokines were not responsible for the inhibitory activity. The capacity of IL-4 to induce inhibitory activity in stromal cell layers suggests that IL-4 may play a role in the regulation of hematopoiesis.

7/7/9 (Item 3 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
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05751291 90198404

Expression of tumor necrosis factor-alpha and transforming growth factor-beta 1 in acute liver injury.

Czaja MJ; Flanders KC; Biempica L; Klein C; Zern MA; Weiner FR

Albert Einstein College of Medicine, Liver Research Center, New York, New York 10461.

Growth Factors (SWITZERLAND) 1989, 1 (3) p219-26, ISSN

0897-7194 Journal Code: AOI

Contract/Grant No.: DK-01792, DK, NIDDK; DK-38484-03, DK, NIDDK

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Tumor necrosis factor-alpha (TNF-alpha) and transforming growth factor-beta 1 (TGF-beta 1) have a number of in vitro functions that could be important in vivo in acute liver injury and repair. Therefore, we investigated these two cytokines in acute liver damage. Northern blots of RNA isolated from rats sacrificed at various time intervals after a single oral dose of CCl4 revealed that TNF-alpha mRNA levels were elevated within 6 hr of CCl4 administration and returned to control values by 24-32 hr. In contrast, TGF-beta 1 mRNA levels started to rise significantly at 24 hr, peaked at 48 hr, and approached baseline levels by 72 hr. Identical changes in TNF-alpha and TGF-beta 1 mRNA levels were also seen with D-galactosamine-induced hepatotoxicity. Immunohistochemical analysis using a TGF-beta 1 antibody demonstrated increased hepatic staining in CCl4-treated rats, at times corresponding to the increases in TGF-beta 1 gene expression. Therefore, there is a differential expression of these cytokines in acute CCl4 and galactosamine hepatotoxicity with an early rise in TNF-alpha, suggesting that this cytokine may affect inflammation and cell toxicity, while TGF-beta 1 peaks later, when it may regulate hepatocyte proliferation and extracellular matrix repair.

7/7/10 (Item 4 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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05739675 90062990

Cell type-specific control of human neuronectin secretion by polypeptide mediators and phorbol ester.

Rettig WJ; Garin-Chesa P

Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021.

J Histochem Cytochem (UNITED STATES) Dec 1989, 37 (12) p1777-86,

ISSN 0022-1554 Journal Code: ID2

Contract/Grant No.: CA-25803-8, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Neuronectin (NEC1) is a human extracellular matrix (ECM) protein expressed with a unique rostrocaudal pattern in white matter of the normal adult central nervous system. In addition, NEC1 is expressed in normal fetal and adult smooth muscle, along certain epithelial-mesenchymal junctions, and transiently in developing fetal cartilage. Region-specific induction of NEC1 is found in dermal wounds and in the reactive stroma of actinic keratoses, psoriatic skin lesions, and a range of malignant tumors. One explanation for these diverse tissue patterns is that cells capable of producing NEC1 are widely distributed in neural and mesenchymal tissues, but they become NEC1 producers only when induced by region-specific differentiation signals. In this study, we used cultured human cells to show that several regulatory polypeptides, including fibroblast growth factors, tumor necrosis factor, platelet-derived growth factor, nerve growth factor, and transforming growth factor-beta (TGF-beta), as well as 12-O-tetradecanoyl phorbol-13-acetate (TPA), modulate NEC1 secretion, with distinct patterns of inducing and inhibitory activities in different neural and mesenchymal cell types. TPA and TGF-beta act both as inducers and inhibitors of NEC1 secretion, depending on the



target cell. These effects are specific for NEC1 and are not seen for several other secreted and membrane proteins studied. We suggest that NEC1 expression comes under different modes of extrinsic control in different cell lineages and in response to tissue injury and neoplasia.

7/7/11 (Item 5 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
(c) format only 1999 Dialog Corporation. All rts. reserv.

05717001 89278018

Modulation of actin mRNAs in cultured vascular cells by matrix components and **TGF-beta 1**.

Kocher O; Madri JA  
Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06510.

In Vitro Cell Dev Biol (UNITED STATES) May 1989, 25 (5) p424-34,  
ISSN 0883-8364 Journal Code: HEQ

Contract/Grant No.: HL-RO1-28373, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Alpha-smooth muscle actin is currently considered a marker of smooth muscle cell differentiation. However, during various physiologic and pathologic conditions, it can be expressed, sometimes only transiently, in a variety of other cell types, such as cardiac and skeletal muscle cells, as well as in nonmuscle cells. In this report, the expression of actin mRNAs in cultured rat capillary endothelial cells (RFCs) and aortic smooth muscle cells (SMCs) has been studied by Northern hybridization in two-dimensional cultures seeded on individual **extracellular matrix** proteins and in three-dimensional type I collagen gels. In two-dimensional cultures, in addition to cytoplasmic actin mRNAs which are normally found in endothelial cell populations, RFCs expressed alpha-smooth muscle (SM) actin mRNA at low levels. alpha-SM actin mRNA expression is dramatically enhanced by **TGF-beta 1**. In addition, double immunofluorescence staining with anti-vWF and anti-alpha-SM-1 (a monoclonal **antibody** to alpha-SM actin) shows that RFCs co-express the two proteins. In three dimensional cultures, RFCs still expressed vWF, but lost staining for alpha-SM actin, whereas alpha-SM actin mRNA became barely detectable. In contrast to two-dimensional cultures, the addition of **TGF-beta 1** to the culture media did not enhance alpha-SM actin mRNA in three-dimensional cultures, whereas it induced rapid capillary tube formation. Actin mRNA expression was modulated in SMCs by **extracellular matrix** components and **TGF-beta 1** with a pattern very different from that of RFCs. Namely, the comparison of RFCs with other cell types such as bovine aortic endothelial cells shows that co-expression of endothelial and smooth muscle cell markers is very unique to RFCs and occurs only in particular culture conditions. This could be related to the capacity of these microvascular endothelial cells to modulate their phenotype in physiologic and pathologic conditions, particularly during angiogenesis, and could reflect different embryologic origins for endothelial cell populations.

7/7/12 (Item 1 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02723297

Reissue

POLYPEPTIDE CARTILAGE-INDUCING FACTORS FOUND IN BONE

[Mixture of transforming growth factor, carriers and polypeptide]

PATENT NO.: RE35,694

ISSUED: December 16, 1997 (19971216)

INVENTOR(s): Seyedin, Saeid, Saratoga, CA (California), US (United States of America)

Thomas, Thomas, West Roxbury, MA (Massachusetts), US (United States of America)  
Bentz, Hanne, Newark, CA (California), US (United States of America)  
Ellingsworth, Larry, San Jose, CA (California), US (United States of America)  
Armstrong, Rosa, Palo Alto, CA (California), US (United States of America)  
ASSIGNEE(s): Celtrix Pharmaceuticals, Inc , (A U.S. Company or Corporation)  
, Santa Clara, CA (California), US (United States of America)  
[Assignee Code(s): 28382]  
APPL. NO.: 8-257,472  
FILED: June 09, 1994 (19940609)  
Reissue (first reissue) of patent no.: 4,843,063, issued: June 27, 1989 (19890627), serial no.: 7-204,173, filed: June 08, 1988 (19880608)

#### CROSS-REFERENCE TO RELATED APPLICATION

This application is a (italic start) continuation of application Ser. No. 07-664,766, filed Mar. 5, 1991 abandoned which is a RE of 07-204,173, filed Jun. 8, 1988, now U.S. Pat. No. 4,843,063, which is a (italic end) continuation of copending U.S. Ser. No. 129,864, filed Dec. 10, 1987, now U.S. Pat. No. 4,774,322, issued Sept. 27, 1988, which is a continuation of U.S. Ser. No. 767,144, filed Aug. 19, 1985, now abandoned, which is a continuation-in-part of U.S. Ser. No. 630,938, filed July 16, 1984, now abandoned.

FULL TEXT: 796 lines

#### ABSTRACT

Two proteins that are found in bone and that have in vivo chondrogenic/osteogenic activity in combination with a co-factor are described. Both proteins also were active in combination with EGF in the in vitro **TGF- beta** assay. Each has a molecular weight of approximately 26,000 daltons by SDS-PAGE. Each is reduced to a single polypeptide indicating that the proteins are probably homodimers. One has an N-terminal sequence identical to that of human placenta-derived **TGF- beta** whereas the other has an N-terminal sequence that is different from that of **TGF- beta** derived from human placenta. The two proteins may be purified to homogeneity using RP-HPLC or acetic acid-urea gel electrophoresis.

We claim: [ 1. A method of promoting proliferation of cells in an animal comprising

administering to the animal a composition comprising a **TGF-beta** activating agent, a pharmaceutically acceptable carrier, and a polypeptide that (a) is found in mammalian bone, (b) is active in the **TGF-beta** assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide and activating agent are present in amounts sufficient to promote bone growth. ] [ 2. The method of claim 1 wherein each chain of said dimer has the following N-terminal sequence: Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp-. ] [ 3. A method for inducing cartilage and bone formation for repairing, replacing, and or augmenting cartilage and bone tissue in animals, which method comprises:

administering to a mammal in need of bone repair, replacement, or augmentation a composition comprising a **TGF-beta** activating agent, a pharmaceutically acceptable excipient, and a polypeptide the (a) is found in mammalian bone, (b) is active in the **TGF-beta** assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide and activating agent are present in amounts sufficient to promote bone growth. ] [ 4. The method of claim 3 wherein each chain of

said dimer has the following N-terminal sequence:  
Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-

Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp-. ] [ 5. A method for treating osteoporosis or osteopetrosis, which comprises:

systemically administering an effective amount of a composition comprising a **TGF-beta** activating agent, a pharmaceutically acceptable excipient, and a polypeptide that (a) is found in mammalian bone, (b) is active in the **TGF-beta** assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide and activating agent are present in amounts sufficient to promote bone growth. ] [ 6. The method of claim 5 wherein each chain of said dimer has the following N-terminal sequence: Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp-. ] [ 7. The method of claim 3 wherein each chain of said dimer has the following N-terminal sequence: Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pro-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp. ] [ 8. The method of claim 5 wherein each chain of said dimer has the following N-terminal sequence: Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pr

o-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp. ] (*italic start*) 9. A method for inducing cartilage and bone formation for repairing, replacing, and/or augmenting cartilage and bone tissue in animals, which method comprises administering to an animal in need of bone repair, replacement, or augmentation a composition comprising a **TGF-beta** activating agent, a pharmaceutically acceptable excipient, and a polypeptide that (a) is found in mammalian bone, (b) is active in the **TGF-beta** assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide and activating agent are present in amounts sufficient to promote bone growth. (*italic end*) (*italic start*) 10. The method of claim 9 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp (**TGF-beta** 1). (*italic end*) (*italic start*) 11. The method of claim 9 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pr o-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp

(**TGF-beta** -2). (*italic end*) (*italic start*) . A method for treating osteoporosis or osteopetrosis, which comprises systemically administering to an individual in need of such treatment an effective amount of a composition comprising a **TGF-beta** activating agent, a pharmaceutically acceptable excipient, and a polypeptide that (a) is found in mammalian bone, (b) is active in the **TGF-beta** assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide and activating agent are present in amounts sufficient to promote bone growth. (*italic end*) (*italic start*) 13. The method of claim 12 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp (**TGF-beta** -1).

(*italic end*) (*italic start*) 14. The method of claim 12 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pr o-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp (**TGF-beta** -2).

(*italic end*) (*italic start*) 15. A method for inducing cartilage and bone formation for repairing, replacing, and/or augmenting cartilage and bone tissue in animals, which method comprises administering to an animal in need of bone repair, replacement, or augmentation a composition comprising a pharmaceutically acceptable excipient and a polypeptide that (a) is found

in mammalian bone, (b) is active in the TGF- beta assay, (c) is a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide is

present in an amount sufficient to promote bone growth. (italic end) (italic start) 16. The method of claim 15 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp (TGF beta -1).

(italic end) (italic start) 17. The method of claim 15 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pro-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp (TGF beta -2).

(italic end) (italic start) 18. A method for treating osteoporosis or osteopetrosis, which comprises systemically administering to an individual in need of such treatment an effective amount of a composition comprising a pharmaceutically acceptable excipient and a polypeptide that (a) is found in mammalian bone, (b) is active in the TGF- beta assay, (c) is

a co-factor for inducing cartilage formation in vivo, (d) is a dimer having an approximate molecular weight of 26,000 daltons as determined by SDS-PAGE, and (e) is substantially pure, wherein said polypeptide is present in an amount sufficient to promote bone growth. (italic end) (italic start) 19. The method of claim 18 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Thr-Asn-Tyr-Cys-Phe-Ser-(Ser)-Thr-Glu-Lys-Asn-Cys-Cys-Val-Arg-Gln-Leu-Tyr-Ile-Asp-Phe-Arg-Lys-Asp-Leu-Gly-Trp

(TGF beta -1). (italic end). I added zero The method of claim 18 wherein each chain of said dimer has the following N-terminal sequence:

Ala-Leu-Asp-Ala-Ala-Tyr-Cys-Phe-Arg-Asn-Val-Gln-Asp-Asn-Cys-Cys-Leu-Arg-Pro-Leu-Tyr-Ile-Asp-Phe-Lys-Arg-Asp-Leu-Gly-Trp (TGF beta -2).

(italic end)

? s s1 and cirrhosis

1282 S1

124987 CIRRHOSIS

S8 97 S1 AND CIRRHOSIS

? s s8 and (vivo or therap? or treat?)

Processing

Processing

97 S8

882941 VIVO

4537499 THERAP?

5329187 TREAT?

S9 92 S8 AND (VIVO OR THERAP? OR TREAT?)

? s s1 and glomerulonephritis

1282 S1

56794 GLOMERULONEPHRITIS

S10 229 S1 AND GLOMERULONEPHRITIS

? rd s10

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>>>Duplicate detection is not supported for File 653.

>>>Duplicate detection is not supported for File 654.

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...examined 50 records (100)

...examined 50 records (150)

...examined 50 records (200)

...completed examining records

S11 170 RD S10 (unique items)

? s s1 and (ards or respiratory(w)distress)

1282 S1

8422 ARDS

748012 RESPIRATORY

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          93151  DISTRESS
          56715  RESPIRATORY(W)DISTRESS
S12      60  S1 AND (ARDS OR RESPIRATORY(W)DISTRESS)
? rd s12
>>>Duplicate detection is not supported for File 652.
>>>Duplicate detection is not supported for File 653.
>>>Duplicate detection is not supported for File 654.

>>>Records from unsupported files will be retained in the RD set.
...examined 50 records  (50)
...completed examining records
      S13      58  RD S12 (unique items)
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s14/3/all

14/3/1 (Item 1 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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03019877

Utility  
CELL-CYCLE REGULATORY PROTEINS, AND USES RELATED THERETO

PATENT NO.: 5,968,821  
ISSUED: October 19, 1999 (19991019)  
INVENTOR(s): Beach, David H., Huntington Bay, NY (New York), US (United States of America)  
Demetrick, Douglas J., E. Northport, NY (New York), US (United States of America)  
Serrano, Manuel, Mill Neck, NY (New York), US (United States of America)  
Hannon, Gregory J., Huntington, NY (New York), US (United States of America)  
ASSIGNEE(s): Cold Spring Harbor Laboratories, Inc , (A U.S. Company or Corporation), Cold Spring Harbor, NY (New York), US (United States of America)  
APPL. NO.: 8-893,274  
FILED: July 15, 1997 (19970715)

#### RELATED APPLICATIONS

This application is a continuation application of Ser. No. 08-306,511 filed on Sep. 14, 1994, which is a continuation-in-part of U.S. Ser. No. 08-248,812 filed May 25, 1994 and entitled "Cell-cycle Regulatory Protein, and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 08-227,371 filed Apr. 14, 1994 and entitled "Cell-cycle Regulatory Protein, and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 08-154,915 filed Nov. 18, 1993 and entitled "Cyclin Complex Rearrangements and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 07-991,997 filed Dec. 17, 1992 and entitled "Cyclin Complex Rearrangements and Uses Related Thereto", abandoned, which is a continuation-in-part of U.S. Ser. No. 07-963,308 filed Oct. 16, 1992 and entitled "D-Type Cyclin and Uses Related Thereto". The teachings of U.S. Ser. Nos. 08-248,812, 08-227,371, 08-154,915, 07-991,997, 07-963,308 and related PCT publication US93-09945 are incorporated herein by reference.

#### FUNDING

Work described herein was supported by National Institutes NIH Grant Nos. ROI GM39620, ROI CA63518 and ROI CA68040 of Health Grant and the Howard Hughes Medical Institute. The United States Government has certain rights in the invention.

FULL TEXT: 3685 lines

14/3/2 (Item 2 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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03019817

Utility

UBIQUITIN CONJUGATING ENZYMES

PATENT NO.: 5,968,761  
ISSUED: October 19, 1999 (19991019)  
INVENTOR(s): Rolfe, Mark, Newton Upper Falls, MA (Massachusetts), US  
(United States of America)  
Chiu, Maria Isabel, Boston, MA (Massachusetts), US (United  
States of America)  
Cottarel, Guillaume, West Roxbury, MA (Massachusetts), US  
(United States of America)  
Berlin, Vivian, Dunstable, MA (Massachusetts), US (United  
States of America)  
Damagnez, Veronique, Cambridge, MA (Massachusetts), US  
(United States of America)  
Draetta, Giulio, Winchester, MA (Massachusetts), US (United  
States of America)  
ASSIGNEE(s): Mitotix, Inc , (A U.S. Company or Corporation), Cambridge, MA  
(Massachusetts), US (United States of America)  
[Assignee Code(s): 36847]  
APPL. NO.: 8-486,663  
FILED: June 07, 1995 (19950607)

RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Ser. No. 08-250,795, filed May 27, 1994 entitled "Immunosuppressant Target Proteins", and is a continuation-in-part of U.S. Ser. No. 08-305,520, filed Sep. 13, 1994, now U.S. Pat No. 5,744,343, entitled "Ubiquitin Conjugating Enzymes", which is a continuation-in-part of U.S. Ser. No. 08-247,904, filed May 23, 1994 entitled "Human Ubiquitin Conjugating Enzyme", which is a continuation-in-part of U.S. Ser. No. 08-176,937, filed Jan. 4, 1994 now abandoned, entitled "Assay and Reagents for Detecting Inhibitors of Ubiquitin-dependent Degredation of Cell Cycle Regulatory Proteins", the specification of which are incorporated by reference herein.

FULL TEXT: 5119 lines

14/3/3 (Item 3 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

03012658

Utility  
CELL-CYCLE REGULATORY PROTEINS, AND USES RELATED THERETO

PATENT NO.: 5,962,316  
ISSUED: October 05, 1999 (19991005)  
INVENTOR(s): Beach, David H., Huntington Bay, NY (New York), US (United  
States of America)  
Demetrick, Douglas J., Northport, NY (New York), US (United  
States of America)  
Serrano, Manuel, Mill Neck, NY (New York), US (United States  
of America)  
Hannon, Gregory J., Huntington, NY (New York), US (United  
States of America)  
ASSIGNEE(s): Cold Spring Harbor Laboratory, (A U.S. Company or Corporation)  
, Cold Spring Harbor, NY (New York), US (United States of  
America)  
[Assignee Code(s): 797]  
APPL. NO.: 8-306,511  
FILED: September 14, 1994 (19940914)

RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Ser. No. 08-248,812 filed May 25, 1994 and entitled "Cell-cycle Regulatory Protein, and Uses

Related Thereto", which is a continuation-in-part of U.S. Ser. No. 08-227,371 filed Apr. 14, 1994 and entitled "Cell-cycle Regulatory Protein, and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 08-154,915 filed Nov. 18, 1993 and entitled "Cyclin Complex Rearrangements and Uses Related Thereto", which is a continuation-in-part of U.S. Ser. No. 07-991,997 filed Dec. 17, 1992 and entitled "Cyclin Complex Rearrangements and Uses Related Thereto", now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-963,308 filed Oct. 16, 1992 and entitled "D-Type Cyclin and Uses Related Thereto". The teachings of U.S. Ser. Nos. 08-248,812, 08-227,371, 08-154,915, 07-991,997, 07-963,308 and related PCT publication US 93-09945 are incorporated herein by reference.

#### FUNDING

Work described herein was supported by National Institutes under NIH Grant Nos. R01 GM39620 and R01 CA63518 of Health Grant and the Howard Hughes Medical Institute. The United States Government has certain rights in the invention.

FULL TEXT: 3628 lines

14/3/4 (Item 4 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
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03008923

Utility  
OCULAR **THERAPY** IN KERATOCONJUNCTIVITIS SICCA USING TOPICALLY APPLIED ANDROGENS OF TGF-**BETA**.

PATENT NO.: 5,958,912  
ISSUED: September 28, 1999 (19990928)  
INVENTOR(s): Sullivan, David A., Acton, MA (Massachusettes), US (United States of America)  
ASSIGNEE(s): The Schepens Eye Research Institute, Inc , (A U.S. Company or Corporation), Boston, MA (Massachusetts), US (United States of America)  
[Assignee Code(s): 39547]  
APPL. NO.: 8-971,768  
FILED: November 17, 1997 (19971117)

This application is a continuation-in-part of Sullivan, U.S. patent application Ser. No. 08-477,301, filed Jun. 7, 1995, now U.S. Pat. No. 5,688,765 which was a continuation-in-part of Sullivan, U.S. patent application Ser. No. 08-124,842, filed Sep. 21, 1993, now U.S. Pat. No. 5,620,921, which was a continuation under 37 CFR 1.62 of U.S. patent application Ser. No. 07-871,657, filed Apr. 21, 1992, now abandoned the whole of which are hereby incorporated by reference herein.

#### GOVERNMENT RIGHTS

Part of the work leading to this invention was made with United States Government funds under Grant No. EY05612 from the National Institutes of Health. Therefore, the U.S. Government has certain rights in this invention.

FULL TEXT: 1278 lines

14/3/5 (Item 5 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.



03005319

Utility

NUCLEIC ACIDS ENCODING PROTEINS FOR EARLY LIVER DEVELOPMENT

PATENT NO.: 5,955,594  
ISSUED: September 21, 1999 (19990921)  
INVENTOR(s): Mishra, Lopa, 6910 Oakridge Ave., Bethesda, MD (Maryland), US  
(United States of America), 20815  
[Assignee Code(s): 68000]  
APPL. NO.: 8-841,349  
FILED: April 30, 1997 (19970430)  
FULL TEXT: 3807 lines

14/3/6 (Item 6 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

03001627

Utility

DNA ENCODING THE CHEMOTACTIC CYTOKINE III

PATENT NO.: 5,952,197  
ISSUED: September 14, 1999 (19990914)  
INVENTOR(s): Ni, Jian, Rockville, MD (Maryland), US (United States of  
America)  
Gentz, Reiner, Silver Spring, MD (Maryland), US (United States  
of America)  
Yu, Guo-Liang, Darnestown, MD (Maryland), US (United States of  
America)  
Su, Jeffrey, Gaithersburg, MD (Maryland), US (United States of  
America)  
Dillon, Patrick J., Gaithersburg, MD (Maryland), US (United  
States of America)  
ASSIGNEE(s): Human Genome Sciences, Inc , (A U.S. Company or Corporation),  
Rockville, MD (Maryland), US (United States of America)  
[Assignee Code(s): 38350]  
APPL. NO.: 8-812,003  
FILED: March 05, 1997 (19970305)

This application claims benefit of 35 U.S.C. section 120 based on  
copending U.S. Provisional Application Ser. No. 60-013,609, filed on Mar.  
5, 1996.

FULL TEXT: 2170 lines

14/3/7 (Item 7 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02977004

Utility

**ANTIBODIES TO GROWTH DIFFERENTIATION FACTOR-12**

PATENT NO.: 5,929,213  
ISSUED: July 27, 1999 (19990727)  
INVENTOR(s): Lee, Se-Jin, Baltimore, MD (Maryland), US (United States of  
America)  
Esquela, Aurora F., Baltimore, MD (Maryland), US (United  
States of America)  
ASSIGNEE(s): The Johns Hopkins University School of Medicine, (A U.S.  
Company or Corporation), Baltimore, MD (Maryland), US (United  
States of America)  
[Assignee Code(s): 39884]  
APPL. NO.: 8-765,662

FILED: April 28, 1997 (19970428)  
PCT: PCT-US95-08745 (WO 95US8745)  
Section 371 Date: April 28, 1997 (19970428)  
Section 102(e) Date: April 28, 1997 (19970428)  
Filing Date: July 12, 1995 (19950712)  
Publication Number: WO96-02559 (WO 962559)  
Publication Date: February 01, 1996 (19960201)

CROSS REFERENCE TO RELATED APPLICATIONS

This is a 371 of PCT-US95-08745, filed Jul. 12, 1995, which is a continuation-in-part of application Ser. No. 08-311,370, filed Sep. 26, 1994, now abandoned, which is a conrica), 98105-2313

[Assignee Code(s): 68000]

APPL. NO.: 8-710,040  
FILED: September 10, 1996 (19960910)

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 08-488,722 filed Jun. 8, 1995, now abandoned, which is a continuation-in-part of prior U.S. application Ser. No. 08-221,365 filed Mar. 31, 1994, now abandoned.

FULL TEXT: 1396 lines

14/3/34 (Item 34 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02644652

Utility  
METHODS FOR **TREATING** DISORDERS BY ADMINISTERING RADIO FREQUENCY  
SIGNALS CORRESPONDING TO GROWTH FACTORS

PATENT NO.: 5,626,617  
ISSUED: May 06, 1997 (19970506)  
INVENTOR(s): Brewitt, Barbara, 6812 Woodlawn Ave. NE., Seattle, WA  
(Washington), US (United States of America), 98115-5420  
[Assignee Code(s): 68000]  
APPL. NO.: 8-575,840  
FILED: December 20, 1995 (19951220)

This application is a continuation of U.S. patent application No. 08-221,365, filed on Mar. 31, 1994, now abandoned.

FULL TEXT: 917 lines

14/3/35 (Item 35 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02562682

Utility  
METHODS OF REDUCING SCARRING IN WOUND HEALING  
{Using a benzothioephene compound}

PATENT NO.: 5,550,151  
ISSUED: August 27, 1996 (19960827)  
INVENTOR(s): Cullinan, George J., Trafalgar, IN (Indiana), US (United States of America)  
ASSIGNEE(s): Eli Lilly Company, (A U.S. Company or Corporation), Indianapolis, IN (Indiana), US (United States of America)  
[Assignee Code(s): 49800]  
APPL. NO.: 8-293,851

FILED: August 22, 1994 (19940822)  
FULL TEXT: 618 lines

14/3/36 (Item 36 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02492661

Utility

CONSTRUCTION AND USE OF SYNTHETIC CONSTRUCTS ENCODING SYNDECAN

PATENT NO.: 5,486,599  
ISSUED: January 23, 1996 (19960123)  
INVENTOR(s): Saunders, Scott, Boston, MA (Massachusetts), US (United States of America)  
Bernfield, Merton, Boston, MA (Massachusetts), US (United States of America)  
Kato, Masato, Boston, MA (Massachusetts), US (United States of America)  
ASSIGNEE(s): Children's Medical Center Corporation, (A U.S. Company or Corporation), Boston, MA (Massachusetts), US (United States of America)  
The Board of Trustees of the Leland Stanford Junior University, (A U.S. Company or Corporation), Palo Alto, CA (California), US (United States of America)  
[Assignee Code(s): 10709; 49136]  
APPL. NO.: 8-78,683  
FILED: June 17, 1993 (19930617)

REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 07-757,654 filed Sep. 6, 1991, now abandoned, and is a continuation-in-part of U.S. patent application Ser. No. 07-856,869 filed Mar. 24, 1992, now abandoned, which is a continuation-in-part of application Ser. No. 07-746,797 filed Aug. 12, 1991, now abandoned, which is a continuation-in-part of application Ser. No. 07-331,585 filed Mar. 29, 1989, now abandoned. All of the above-referenced patent applications are hereby incorporated by reference.

Work leading to the present invention was supported in part by a National Institutes of Health grant. The government has rights in this invention as a result of this support.

FULL TEXT: 4214 lines

14/3/37 (Item 37 from file: 654)  
DIALOG(R)File 654:US Pat.Full.  
(c) format only 1999 The Dialog Corp. All rts. reserv.

02174133

Utility

METHODS FOR CONTROLLING HUMAN ENDOTHELIAL CELL PROLIFERATION AND EFFECTOR FUNCTIONS USING ONCOSTATIN M  
[Supression of cytokine-stimulated expression of MHC antigens]

PATENT NO.: 5,202,116  
ISSUED: April 13, 1993 (19930413)  
INVENTOR(s): Brown, Thomas J., Poulsbo, WA (Washington), US (United States of America)  
Gladstone, Paul R., Seattle, WA (Washington), US (United States of America)  
ASSIGNEE(s): Oncogen, (A U.S. Company or Corporation), Seattle, WA

(Washington), US (United States of America)  
[Assignee Code(s): 14317]

APPL. NO.: 7-335,399

FILED: April 10, 1989 (19890410)

FULL TEXT: 1178 lines

TEM:OS - DIALOG OneSearch

File 5:Biosis Previews(R) 1969-2003/Aug W1

(c) 2003 BIOSIS

File 73:EMBASE 1974-2003/Aug W1

(c) 2003 Elsevier Science B.V.

\*File 73: Alert feature enhanced for multiple files, duplicates removal, customized scheduling. See HELP ALERT.

File 155:MEDLINE(R) 1966-2003/Aug W2

(c) format only 2003 The Dialog Corp.

\*File 155: Medline has been reloaded and accession numbers have changed. Please see HELP NEWS 155.

File 399:CA SEARCH(R) 1967-2003/UD=13907

(c) 2003 American Chemical Society

\*File 399: Use is subject to the terms of your user/customer agreement. Alert feature enhanced for multiple files, etc. See HELP ALERT.

Set Items Description

? s (interstitial(W)lung(W)fibrosis)

145856 INTERSTITIAL

1061551 LUNG

197668 FIBROSIS

S1 214 (INTERSTITIAL(W)LUNG(W)FIBROSIS)

? s s1 and ards

214 S1

10473 ARDS

S2 4 S1 AND ARDS

? rd s2

...completed examining records

S3 2 RD S2 (unique items)

? t s3/7/all

3/7/1 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.

13211095 BIOSIS NO.: 200100418244

Granulocyte-macrophage-colony-stimulating-factor (GM-CSF) expression in the human lung: An immunohistochemical analysis.

AUTHOR: Burg J(a); Bittinger F(a); Krump-Konvalinkova V(a); Kirkpatrick C J (a)

AUTHOR ADDRESS: (a)Institute of Pathology, Johannes Gutenberg University of Mainz, Mainz\*\*Germany

JOURNAL: Pathology Research and Practice 197 (5):p334 2001

MEDIUM: print

CONFERENCE/MEETING: 85th Meeting of the German Society of Pathology Muenster, Germany June 06-09, 2001

ISSN: 0344-0338

RECORD TYPE: Citation

LANGUAGE: English

SUMMARY LANGUAGE: English

3/7/2 (Item 2 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.

11385474 BIOSIS NO.: 199800166806

Soluble complement receptor type 1 (CD35) in bronchoalveolar lavage of inflammatory lung diseases.

AUTHOR: Hamacher J; Sadallah S; Schifferli J A; Villard J; Nicod L P(a)

AUTHOR ADDRESS: (a)Pulmonary Div., Hopital Cantonal Univ., CH-1211 Geneva 14\*\*Switzerland

JOURNAL: European Respiratory Journal 11 (1):p112-119 Jan., 1998

ISSN: 0903-1936  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

**ABSTRACT:** Complement receptor type 1 (CR1) (CD35; C3b/C4b receptor) is a transmembrane protein of many haematopoietic cells. Once cleaved, soluble complement receptor type 1 (sCR1) exerts opposite effects as a powerful inhibitor of complement. This study addressed both the question of whether sCR1 was found in bronchoalveolar lavage (BAL) of normals and patients with various inflammatory disease, and its possible origin. In this retrospective study covering specimen and clinical data of 124 patients with acute and chronic inflammatory lung pathologies, BAL supernatants were analysed by enzyme-linked immunosorbent assay technique for sCR1. Correlations were made between the sCR1 levels obtained and the constituents of BAL. Human alveolar macrophages were cultivated in order to determine their secretory capacity of sCR1. Alveolar macrophages from normal subjects were shown to release sCR1 in vitro. In addition, sCR1 was present in BAL of normal controls and was significantly increased in acute inflammatory lung diseases such as acute respiratory distress syndrome (ARDS), bacterial and Pneumocystis carinii pneumonia, as well as in chronic inflammatory diseases such as **interstitial lung fibrosis** and sarcoidosis. In BAL of **ARDS**, bacterial, and P. carinii pneumonia, there was a good correlation between sCR1 and the absolute neutrophil counts. In sarcoidosis, a correlation was found with BAL lymphocyte counts. Serum sCR1 was not increased in patients compared to controls. Soluble complement receptor type 1 (sCR1) is found in the bronchoalveolar lavage in health as well as in acute and chronic inflammatory disease. Alveolar macrophages are capable of releasing sCR1 in vitro and may be the main physiological source of sCR1 in the alveoli. The good correlation between sCR1 and the absolute neutrophil or lymphocyte numbers in bronchoalveolar lavage of inflammatory diseases suggests a predominant role of leucocytes for the release of sCR1 in such conditions. The release of this inhibitor of complement may be crucial to control and reduce complement activation and thus prevent lung injury.

? ds

Set	Items	Description
S1	214	(INTERSTITIAL(W) LUNG(W) FIBROSIS)
S2	4	S1 AND ARDS
S3	2	RD S2 (unique items)

? s s1 and adult(W)respiratory

214	S1
4629926	ADULT
924145	RESPIRATORY
17485	ADULT(W)RESPIRATORY

S4	1	S1 AND ADULT(W)RESPIRATORY
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? t s4/7/all

4/7/1 (Item 1 from file: 5)

DIALOG(R)File 5: Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.

13211095 BIOSIS NO.: 200100418244

Granulocyte-macrophage-colony-stimulating-factor (GM-CSF) expression in the human lung: An immunohistochemical analysis.

AUTHOR: Burg J(a); Bittinger F(a); Krump-Konvalinkova V(a); Kirkpatrick C J (a)

AUTHOR ADDRESS: (a) Institute of Pathology, Johannes Gutenberg University of Mainz, Mainz\*\*Germany

JOURNAL: Pathology Research and Practice 197 (5):p334 2001

MEDIUM: print

CONFERENCE/MEETING: 85th Meeting of the German Society of Pathology

Muenster, Germany June 06-09, 2001

• ISSN: 0344-0338

RECORD TYPE: Citation

LANGUAGE: English

SUMMARY LANGUAGE: English

?

? s (ARDS) and (tgfbeta or tgf(w)beta)

Processed 10 of 22 files ...

Processing

Completed processing all files

18839 ARDS

9789 TGF BETA

126654 TGF

3272929 BETA

92709 TGF(W) BETA

S4 42 (ARDS) AND (TGF BETA OR TGF(W) BETA)

? rd s4

>>>Record 266:199550 ignored; incomplete bibliographic data, not retained -  
in RD set

>>>Record 266:199549 ignored; incomplete bibliographic data, not retained -  
in RD set

...completed examining records

S5 20 RD S4 (unique items)

? t s5/3/all

5/3/1 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.

14231352 BIOSIS NO.: 200300225381

The acute respiratory distress syndrome: A role for transforming growth  
factor-beta1.

AUTHOR: Fahy Ruairi J(a); Lichtenberger Frank; McKeegan Christine B; Nuovo  
Gerard J; Marsh Clay B; Wewers Mark D

AUTHOR ADDRESS: (a)The Davis Heart and Lung Research Institute, 473 West  
12th Avenue, 201, Columbus, OH, 43210-1252, USA\*\*USA E-Mail:  
fahy-1@medctr.osu.edu

JOURNAL: American Journal of Respiratory Cell and Molecular Biology 28 (4  
) :p499-503 April 2003 2003

MEDIUM: print

ISSN: 1044-1549

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

5/3/2 (Item 2 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.

14117115 BIOSIS NO.: 200300111144

Roles for insulin-like growth factor I and transforming growth factor-beta  
in fibrotic lung disease.

AUTHOR: Krein Peter M; Winston Brent W(a)

AUTHOR ADDRESS: (a)Departments of Medicine, Biochemistry and Molecular  
Biology, and Critical Care Medicine, Health Sciences Center, University  
of Calgary, 3330 Hospital Dr NW, Room 1843, Calgary, AB, T2N 4N1, Canada  
\*\*Canada E-Mail: bwinston@ucalgary.ca

JOURNAL: Chest 122 (6 Suppl.):p289S-293S December 2002 2002

MEDIUM: print

ISSN: 0012-3692

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

5/3/3 (Item 3 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.



13845032 BIOSIS NO.: 200200473853

TGF-beta alters steroid-induced expression of sodium channels  
in distal lung epithelia.

AUTHOR: Jain Lucky(a); Chen Xi-Juan(a); Eaton Douglas C(a)

AUTHOR ADDRESS: (a)Pediatrics and Physiology, Emory University, Atlanta, GA

\*\*USA

JOURNAL: Pediatric Research 51 (4 Part 2):p474A April, 2002

MEDIUM: print

CONFERENCE/MEETING: Annual Meeting of the Pediatric Societies' Baltimore,  
MD, USA May 04-07, 2002

ISSN: 0031-3998

RECORD TYPE: Citation

LANGUAGE: English

5/3/4 (Item 4 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.

13160861 BIOSIS NO.: 200100368010

TNF and angiostatin but not TGF-beta1 are mediators endothelial cells in  
bronchoalveolar lavages of ARDS patients.

AUTHOR: Hamacher J(a); Ricou B; Dunant Y; Lijnen H R; Grau G E; Wendel A(a)  
; Suter P M; Lucas R(a)

AUTHOR ADDRESS: (a)Biochemical Pharmacology, University of Konstanz,  
D-78457, Konstanz\*\*Germany

JOURNAL: Naunyn-Schmiedeberg's Archives of Pharmacology 363 (4 Supplement  
) :pR110 2001

MEDIUM: print

CONFERENCE/MEETING: 42nd Spring Meeting of the German Society for  
Experimental and Clinical Pharmacology and Toxicology Mainz, Germany  
March 13-15, 2001

ISSN: 0028-1298

RECORD TYPE: Citation

LANGUAGE: English

SUMMARY LANGUAGE: English

5/3/5 (Item 5 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.

13123403 BIOSIS NO.: 200100330552

Aerosolized perfluorocarbon decreases pulmonary inflammatory response and  
improves gas exchange in piglets with ARDS.

AUTHOR: von der Hardt Katharina(a); Kandler Michael A(a); Schoof Ellen(a);  
Doetsch Joerg(a); Rascher Wolfgang(a)

AUTHOR ADDRESS: (a)Klinik fuer Kinder und Jugendliche, Universitaet  
Erlangen-Nuernberg, Erlangen\*\*Germany

JOURNAL: Pediatric Research 49 (4 Part 2):p46A April, 2001

MEDIUM: print

CONFERENCE/MEETING: Annual Meeting of the Pediatric Academic Societies  
Baltimore, Maryland, USA April 28-May 01, 2001

ISSN: 0031-3998

RECORD TYPE: Citation

LANGUAGE: English

SUMMARY LANGUAGE: English

5/3/6 (Item 6 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.

11361498 BIOSIS NO.: 199800142830

Upregulation of postbacteremic TNF-alpha and IL-1alpha gene expression by  
alveolar hypoxia/reoxygenation in perfused rat lungs.  
AUTHOR: Matuschak George M(a); Munoz Cesar F; Johanns Cheryl A; Rahman  
Rashid; Lechner Andrew J  
AUTHOR ADDRESS: (a)Div. Pulmonol., Saint Louis Univ. Hosp., 3635 Vista Ave.  
at Grand Blvd., Saint Louis, MO 63110-0\*\*USA  
JOURNAL: American Journal of Respiratory and Critical Care Medicine 157 (2  
) :p629-637 Feb., 1998  
ISSN: 1073-449X  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

5/3/7 (Item 7 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
(c) 2003 BIOSIS. All rts. reserv.

09504534 BIOSIS NO.: 199497512904  
Anti-transforming growth factor-beta monoclonal antibodies prevent lung  
injury in hemorrhaged mice.  
AUTHOR: Shenkar Robert; Coulson Walter F; Abraham Edward(a)  
AUTHOR ADDRESS: (a)Div. Pulmonary Sci. and Critical Care Med., Univ. Colo.  
Health Sci. Cent., Box C272, 4200 E. Nin\*\*USA  
JOURNAL: American Journal of Respiratory Cell and Molecular Biology 11 (3  
) :p351-357 1994  
ISSN: 1044-1549  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

5/3/8 (Item 8 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
(c) 2003 BIOSIS. All rts. reserv.

09433654 BIOSIS NO.: 199497442024  
Elevated **TGF-beta** activity in patients with severe **ARDS**  
on extracorporeal life support.  
AUTHOR: Fazzalari Franco(a); Phan Shem; Bonnell Mark; Bliss David; Hirschl  
Ron; Bartlett Robert  
AUTHOR ADDRESS: (a)Dep. Surg., Univ. Mich. Med. Sch., Ann Arbor, MI 48109\*\*  
USA  
JOURNAL: Chest 106 (2 SUPPL.):p64S 1994  
CONFERENCE/MEETING: 60th Annual Scientific Assembly of the American College  
of Chest Physicians New Orleans, Louisiana, USA October 30-November 3,  
1994  
ISSN: 0012-3692  
RECORD TYPE: Citation  
LANGUAGE: English

5/3/9 (Item 1 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2003 Inst for Sci Info. All rts. reserv.

11825811 Genuine Article#: 699QW No. References: 97  
Title: Significant involvement of CCL2 (MCP-1) in inflammatory disorders of  
the lung  
Author(s): Rose CE (REPRINT) ; Sung SSJ; Fu SM  
Corporate Source: Univ Virginia,Sch Med, Div Pulm & Crit Care Med,Box  
800546/Charlottesville//VA/22908 (REPRINT); Univ Virginia,Sch Med, Div  
Pulm & Crit Care Med,Charlottesville//VA/22908; Univ Virginia,Sch Med,  
Div Rheumatol & Immunol,Charlottesville//VA/22908

Journal: MICROCIRCULATION, 2003, V10, N3-4 (JUL), P273-288  
ISSN: 1073-9688 Publication date: 20030700  
Publisher: NATURE PUBLISHING GROUP, 345 PARK AVE SOUTH, NEW YORK, NY  
10010-1707 USA  
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

5/3/10 (Item 2 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2003 Inst for Sci Info. All rts. reserv.

11712375 Genuine Article#: 686HB No. References: 50  
Title: Elevated levels of plasminogen activator inhibitor-1 in pulmonary edema fluid are associated with mortality in acute lung injury  
Author(s): Prabhakaran P; Ware LB; White KE; Cross MT; Matthay MA; Olman MA (REPRINT)  
Corporate Source: Univ Alabama, Med Ctr, Dept Med, Div Pulm & Crit Care Med, 1900 Univ Blvd, 215 THT/Birmingham//AL/35294 (REPRINT); Univ Alabama, Med Ctr, Dept Med, Div Pulm & Crit Care Med, Birmingham//AL/35294; Univ Alabama, Dept Pediat, Birmingham//AL/35294; Univ Alabama, Dept Pathol, Birmingham//AL/35294; Vanderbilt Univ, Sch Med, Div Allergy Pulm & Crit Care Med, Nashville//TN/37232; Univ Calif San Francisco, Cardiovasc Res Inst, San Francisco//CA/94143  
Journal: AMERICAN JOURNAL OF PHYSIOLOGY-LUNG CELLULAR AND MOLECULAR PHYSIOLOGY, 2003, V285, N1 (JUL), PL20-L28  
ISSN: 1040-0605 Publication date: 20030700  
Publisher: AMER PHYSIOLOGICAL SOC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA  
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

5/3/11 (Item 3 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2003 Inst for Sci Info. All rts. reserv.

11585959 Genuine Article#: 672HG No. References: 70  
Title: Transforming growth factor-beta: A mediator of cell regulation in acute respiratory distress syndrome  
Author(s): Dhainaut JF (REPRINT); Charpentier J; Chiche JD  
Corporate Source: Univ Paris 05, Hop Cochin, Fac Cochin Port Royal, Serv Reanimat Med, Pavillon Cornil/Paris 14//France/ (REPRINT); Univ Paris 05, Hop Cochin, Fac Cochin Port Royal, Serv Reanimat Med, Paris 14//France/  
Journal: CRITICAL CARE MEDICINE, 2003, V31, N4, S (APR), PS258-S264  
ISSN: 0090-3493 Publication date: 20030400  
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA  
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

5/3/12 (Item 4 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2003 Inst for Sci Info. All rts. reserv.

10719322 Genuine Article#: 559LW No. References: 92  
Title: The pulmonary physcian in critical care center dot 6: The pathogenesis of ALI/ARDS  
Author(s): Bellingan GJ (REPRINT)  
Corporate Source: Univ Coll Hosp, Dept Intens Care Med, Ctr Resp Res, Rayne Inst, London WC1E 6JJ//England/ (REPRINT); Univ Coll Hosp, Dept Intens Care Med, Ctr Resp Res, Rayne Inst, London WC1E 6JJ//England/  
Journal: THORAX, 2002, V57, N6 (JUN), P540-546  
ISSN: 0040-6376 Publication date: 20020600  
Publisher: BRITISH MED JOURNAL PUBL GROUP, BRITISH MED ASSOC HOUSE,

TAVISTOCK SQUARE, LONDON WC1H 9JR, ENGLAND  
Language: English Document Type: REVIEW

5/3/13 (Item 5 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2003 Inst for Sci Info. All rts. reserv.

08154852 Genuine Article#: 230DT No. References: 0  
Title: **TGF beta**(1) levels in BAL fluid correlate with  
resolution of **ARDS**.  
Author(s): Dhainaut JF; Thebert D; Marullo S; DinhXuan T; Monchi M; Brunet  
F  
Corporate Source: CHU COCHIN PORT ROYAL,DEPT ICU/PARIS//FRANCE/; CHU COCHIN  
PORT ROYAL,DEPT PHYSIOL/PARIS//FRANCE/; CHU COCHIN PORT ROYAL,DEPT  
PHARMACOL/PARIS//FRANCE/  
Journal: AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE, 1999  
, V159, N3,S (MAR), PA377-A377  
ISSN: 1073-449X Publication date: 19990300  
Publisher: AMER LUNG ASSOC, 1740 BROADWAY, NEW YORK, NY 10019  
Language: English Document Type: MEETING ABSTRACT

5/3/14 (Item 6 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2003 Inst for Sci Info. All rts. reserv.

06280978 Genuine Article#: YF682 No. References: 35  
Title: Cytokines and soluble cytokine receptors in pleural effusions from  
septic and nonseptic patients  
Author(s): Marie C; Losser MR; Fitting C; Kermarrec N; Payen D; Cavallion  
JM (REPRINT)  
Corporate Source: INST PASTEUR,UNITE IMMUNOALLERGIE, 28 RUE DR ROUX/F-75015  
PARIS//FRANCE/ (REPRINT); INST PASTEUR,UNITE IMMUNOALLERGIE/F-75015  
PARIS//FRANCE/; HOP LARIBOISIERE,DEPT ANAESTHESIA/F-75475  
PARIS//FRANCE/; HOP LARIBOISIERE,INTENS CARE UNIT/F-75475  
PARIS//FRANCE/  
Journal: AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE, 1997  
, V156, N5 (NOV), P1515-1522  
ISSN: 1073-449X Publication date: 19971100  
Publisher: AMER LUNG ASSOC, 1740 BROADWAY, NEW YORK, NY 10019  
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

5/3/15 (Item 7 from file: 34)  
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci  
(c) 2003 Inst for Sci Info. All rts. reserv.

02437429 Genuine Article#: LB149 No. References: 0  
Title: TRANSFORMING GROWTH-FACTOR-BETA-1 (**TGF-BETA**-1) IN  
BRONCHOALVEOLAR LAVAGE OF PATIENTS WITH ADULT RESPIRATORY-DISTRESS  
SYNDROME (**ARDS**)  
Author(s): LAWLOR DP; TRICOMI S; HYERS TM  
Corporate Source: ST LOUIS UNIV,SCH MED,DIV PULMONOL & PULM OCCUPAT MED/ST  
LOUIS//MO/63104  
Journal: AMERICAN REVIEW OF RESPIRATORY DISEASE, 1993, V147, N4 (APR), P  
A734  
ISSN: 0003-0805  
Language: ENGLISH Document Type: MEETING ABSTRACT

5/3/16 (Item 1 from file: 65)  
DIALOG(R)File 65:Inside Conferences  
(c) 2003 BLDSC all rts. reserv. All rts. reserv.

02026615    INSIDE CONFERENCE ITEM ID: CN021104054

Infections and the Compensatory Anti-Inflammatory Response During  
ARDS: Plasma and BAL Levels of TGF- $\beta$ . IL-1ra, and  
sTNFR's

CONFERENCE: American College of Chest Physicians: CHEST 1997-Meeting  
CHEST -CHICAGO-, 1997; VOL 112; NUMBER 3 SUPP P: 56S

American College of Chest Physicians, 1997

ISSN: 0012-3692

LANGUAGE: English    DOCUMENT TYPE: Conference Preprinted abstracts and  
programme

CONFERENCE SPONSOR: American College of Chest Physicians

CONFERENCE LOCATION: New Orleans, LA

CONFERENCE DATE: Oct 1997 (199710) (199710)

5/3/17    (Item 1 from file: 73)

DIALOG(R)File 73:EMBASE

(c) 2003 Elsevier Science B.V. All rts. reserv.

06519657    EMBASE No: 1996185319

The role of cytokines in human lung fibrosis

Vaillant P.; Menard O.; Vignaud J.-M.; Martinet N.; Martinet Y.

Fed. Medico-Chirurgicale Pneumologie, CHU de Nancy-Brabois, Allée du

Morvan, 54511 Vandoeuvre-les-Nancy France

Monaldi Archives for Chest Disease ( MONALDI ARCH. CHEST DIS. ) (Italy)

1996, 51/2 (145-152)

CODEN: MACDE    ISSN: 1122-0643

DOCUMENT TYPE: Journal; Article

LANGUAGE: ENGLISH    SUMMARY LANGUAGE: ENGLISH

5/3/18    (Item 2 from file: 73)

DIALOG(R)File 73:EMBASE

(c) 2003 Elsevier Science B.V. All rts. reserv.

05536951    EMBASE No: 1993305050

Cytokines in relation to ICU problems

Wardle E.N.

21 Common Road, North Leigh OX8 6RD United Kingdom

Clinical Intensive Care ( CLIN. INTENSIVE CARE ) (United Kingdom) 1993,

4/4 (183-189)

CODEN: CICAIE    ISSN: 0956-3075

DOCUMENT TYPE: Journal; Review

LANGUAGE: ENGLISH    SUMMARY LANGUAGE: ENGLISH

5/3/19    (Item 1 from file: 144)

DIALOG(R)File 144:Pascal

(c) 2003 INIST/CNRS. All rts. reserv.

15787883    PASCAL No.: 02-0502262

Tumor necrosis factor-  $\alpha$  and angiostatin are mediators of  
endothelial cytotoxicity in bronchoalveolar lavages of patients with acute  
respiratory distress syndrome

HAMACHER Juerg; LUCAS Rudolf; LIJNEN H Roger; BUSCHKE Susanne; DUNANT  
Yves; WENDEL Albrecht; GRAU Georges E; SUTER Peter M; RICOU Bara

Department of Anaesthesiology, Pharmacology, and Surgical Intensive Care,  
University Medical Center, Geneva, Switzerland; Division of Biochemical  
Pharmacology, University of Konstanz, Konstanz, Germany; Cardiovascular  
Research, VIB, University of Leuven, Leuven, Belgium; Department of  
Physiology, Université de la Méditerranée, Marseille, France

Journal: American journal of respiratory and critical care medicine,  
2002, 166 (5) 651-656

Language: English

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5/3/20 (Item 1 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
(c) format only 2003 The Dialog Corp. All rts. reserv.

10435831 96242491 PMID: 8678788  
Cytokines in human lung fibrosis.  
Martinet Y; Menard O; Vaillant P; Vignaud J M; Martinet N  
INSERM U 14, Nancy-Vandoeuvre.  
Archives of toxicology. Supplement. Archiv fur Toxikologie. Supplement (GERMANY) 1996, 18 p127-39, ISSN 0171-9750 Journal Code: 7802567  
Document type: Journal Article; Review; Review, Tutorial  
Languages: ENGLISH  
Main Citation Owner: NLM  
Record type: Completed

?

File 5: Biosis Previews(R) 1969-2003/Aug W1

(c) 2003 BIOSIS

File 5: EMBASE 1974-2003/Aug W1

(c) 2003 Elsevier Science B.V.

\*File 73: Alert feature enhanced for multiple files, duplicates removal, customized scheduling. See HELP ALERT.

File 155: MEDLINE(R) 1966-2003/Aug W2

(c) format only 2003 The Dialog Corp.

\*File 155: Medline has been reloaded and accession numbers have changed. Please see HELP NEWS 155.

File 399: CA SEARCH(R) 1967-2003/UD=13907

(c) 2003 American Chemical Society

\*File 399: Use is subject to the terms of your user/customer agreement. Alert feature enhanced for multiple files, etc. See HELP ALERT.

Set Items Description

? s (interstitial(w)pulmonary(w)fibrosis or IPF or interstitial(w)lung(w)fibrosis)  
and (ARDS or adult(w)respiratory)

145856 INTERSTITIAL

802082 PULMONARY

197668 FIBROSIS

1262 INTERSTITIAL(W) PULMONARY(W) FIBROSIS

2213 IPF

145856 INTERSTITIAL

1061551 LUNG

197668 FIBROSIS

214 INTERSTITIAL(W) LUNG(W) FIBROSIS

10473 ARDS

4629926 ADULT

924145 RESPIRATORY

17485 ADULT(W) RESPIRATORY

S1 72 (INTERSTITIAL(W) PULMONARY(W) FIBROSIS OR IPF OR  
INTERSTITIAL(W) LUNG(W) FIBROSIS) AND (ARDS OR  
ADULT(W) RESPIRATORY)

? rd s1

...examined 50 records (50)

...completed examining records

S2 34 RD S1 (unique items)

? t s2/7/all

2/7/1 (Item 1 from file: 5)

DIALOG(R) File 5: Biosis Previews(R)

(c) 2003 BIOSIS. All rts. reserv.

14117115 BIOSIS NO.: 200300111144

Roles for insulin-like growth factor I and transforming growth factor-beta  
in fibrotic lung disease.

AUTHOR: Krein Peter M; Winston Brent W(a)

AUTHOR ADDRESS: (a) Departments of Medicine, Biochemistry and Molecular  
Biology, and Critical Care Medicine, Health Sciences Center, University  
of Calgary, 3330 Hospital Dr NW, Room 1843, Calgary, AB, T2N 4N1, Canada

\*\*Canada E-Mail: bwinston@ucalgary.ca

JOURNAL: Chest 122 (6 Suppl.):p289S-293S December 2002 2002

MEDIUM: print

ISSN: 0012-3692

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Idiopathic pulmonary fibrosis (IPF) is a lung disease that  
is characterized by epithelial cell damage and areas of denuded basement  
membrane resulting in inflammation, fibroblast proliferation, excessive  
extracellular matrix (ECM) deposition, and remodeling of alveolar gas

exchange units. The progressive loss of lung gas exchange units in patients with **IPF** leads to respiratory failure and eventually to death. While the etiology of this disease is unknown, for many years studies suggested that chronic inflammation was the underlying factor that caused fibroproliferation and structural alterations of the lung. Recent data show that fibroproliferation and fibrosis can occur independently of inflammation, suggesting that **IPF** is a disease caused by a mesenchymal, rather than an immune disorder. Mesenchymal growth factors, including transforming growth factor (TGF)-beta, insulin-like growth factor (IGF)-I, platelet-derived growth factor, connective tissue growth factor, fibroblast growth factors, and keratinocyte growth factors, as well as proinflammatory cytokines such as tumor necrosis factor-alpha and interleukin-1beta, have been shown to be exaggerated in several fibrotic lung disorders including **IPF**, **ARDS**, sarcoidosis, and bronchopulmonary dysplasia, as well as pulmonary manifestations of systemic diseases such as rheumatoid arthritis or progressive systemic sclerosis (scleroderma). We argue that inflammation is required to initiate growth factor production and repair of the damaged alveolar epithelial lining in fibrotic lung diseases and that exaggerated TGF-beta production may be responsible for the fibrotic response seen in diseases such as **IPF**. We recognize the potential role of several growth factors in the fibroproliferative process in the lung, and in this brief report we focus on the possible roles of the growth factors IGF-I and TGF-beta in cell migration, proliferation, and ECM synthesis in patients with **IPF**.

2/7/2 (Item 2 from file: 5)  
DIALOG(R) File 5: Biosis Previews(R)  
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14079231 BIOSIS NO.: 200300073260

The SH-metabolite I of erdosteine, a mucolytic drug, enhances the inhibitory effect of salbutamol on the respiratory burst of neutrophils.

AUTHOR: Dal Sasso M; Bovio C; Culici M; Ponti E; Braga P C(a)

AUTHOR ADDRESS: (a)Dept. of Pharmacology, School of Medicine, Via Vanvitelli 32, 20129, Milan, Italy\*\*Italy E-Mail:

bragapc@mailserver.unimi.it

JOURNAL: Drugs under Experimental and Clinical Research 28 (4):p147-154

2002

MEDIUM: print

ISSN: 0378-6501

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

**ABSTRACT:** Reactive oxygen species (ROS) are a common denominator of airway inflammation associated with chronic obstructive pulmonary disease (COPD) and asthma, as well as with less frequent lung diseases such as idiopathic pulmonary fibrosis (**IPF**), acute respiratory distress syndrome (**ARDS**) and cystic fibrosis (CF). The most frequently administered drugs used to treat these diseases are bronchodilators, antioxidant/antiphlogistic agents and mucoactive drugs. The metabolism of the mucoactive drug erdosteine produces an active metabolite (Met I) with a reducing SH group. In addition to its mucolytic action, Met I also has useful antioxidant activity. The various activities of beta2-agonists include their ability to reduce the respiratory burst of neutrophils and the subsequent release of ROS. beta2-Agonists and mucoactive drugs may be administered to the same patients during the treatment of lung diseases. The aim of this study was to investigate the ability of Met I to potentiate the activity of salbutamol in inhibiting the in vitro respiratory burst of neutrophils by means of chemiluminescence. The combination of Met I 5 and 10 mug/ml with salbutamol 10-5, 10-6 and 10-7 M led to a significant reduction in



respiratory bursts when the neutrophils were stimulated with the soluble stimulant N-formyl-methionyl-leucyl-phenylalanine (fMLP). The combinations of the two drugs that reduced the respiratory bursts when a particulate stimulus (*Candida albicans*) was used were those containing 10-5 M of salbutamol. The reasons for this different behavior remain unclear and raise questions about the specific roles, sites and mechanisms of action of the different types of stimulation undergone by the respiratory airways.

2/7/3 (Item 3 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
(c) 2003 BIOSIS. All rts. reserv.

13873168 BIOSIS NO.: 200200501989

Sulfonyl urea derivatives and their use in the control of interleukin-1 activity.

AUTHOR: Dombroski Mark Anthony; Egger James Frederick  
JOURNAL: Official Gazette of the United States Patent and Trademark Office  
Patents 1261 (2):pNo Pagination Aug. 13, 2002

MEDIUM: e-file

ISSN: 0098-1133

DOCUMENT TYPE: Patent

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A compound of the formula ##STR1## wherein R1 and R2 are as defined above, useful in the treatment and condition selected from the group consisting of meningitis and salpingitis, septic shock, disseminated intravascular coagulation, and/or **adult respiratory** distress syndrome, acute or chronic inflammation, arthritis, cholangitis, colitis, encephalitis, endocarditis, glomerulonephritis, hepatitis, myocarditis, pancreatitis, pericarditis, reperfusion injury, vasculitis, acute and delayed hypersensitivity, graft rejection, and graft-versus-host disease, auto-immune diseases including Type 1 diabetes mellitus and multiple sclerosis, periodonate diseases, **interstitial pulmonary fibrosis**, cirrhosis, systemic sclerosis, keloid formation, tumors which produce IL-1 as an autocrine growth factor, cachexia, Alzheimers disease, percussion injury, depression, atherosclerosis, osteoporosis in a mammal, including a human.

2/7/4 (Item 4 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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13787655 BIOSIS NO.: 200200416476

Role of defenses in inflammatory lung disease.

AUTHOR: Aarbiou Jamil(a); Rabe Klaus F; Hiemstra Pieter S

AUTHOR ADDRESS: (a)Dept. of Pulmonology, Leiden University Medical Center, C3-P, NL-2300 RC, P.O. Box 9600, Leiden\*\*Netherlands E-Mail:

j.aarbiou@lumc.nl

JOURNAL: Annals of Medicine 34 (2):p96-101 2002

MEDIUM: print

ISSN: 0785-3890

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The human airways are protected from invading micro-organisms by the highly efficient innate immune system. Antimicrobial peptides that are produced by inflammatory cells and airway epithelial cells are key elements in this innate immune system. A major subgroup of the antimicrobial peptides is the family of defensins - small non-enzymatic

and cationic peptides. Besides their extensively studied role in antimicrobial defense, recent studies have demonstrated that defensins are also able to modulate inflammatory responses, to stimulate adaptive immunity and contribute to tissue repair. In line with these observations, increased defensin levels were observed in inflammatory lung diseases, such as cystic fibrosis (CF), diffuse panbronchiolitis (DPB), idiopathic pulmonary fibrosis (IPF) and acute respiratory distress syndrome (ARDS), and in infectious diseases. In the past decade much has been learnt about the activity of defensins and there is abundant evidence for their presence in human inflammatory lung disease. Future studies are required to elucidate their role in the pathogenesis of these diseases.

2/7/5 (Item 5 from file: 5)  
DIALOG(R)File 5: Biosis Previews(R)  
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13741202 BIOSIS NO.: 200200370023  
Effect of intratracheal administration of LF-B25 on in vivo proliferation of normal rat and monkey pulmonary epithelial cells.  
AUTHOR: Connolly Kevin Michael(a); Okoye Zebedee; Santiago Gemma; Windle Kathleen; Wojcik Susan; Wong Eling; Fikes James  
AUTHOR ADDRESS: (a) Clinical Operations, Human Genome Sciences Inc., 9410 Key West Avenue, Rockville, MD, 20850\*\*USA  
JOURNAL: FASEB Journal 16 (5):pA953-A954 March 22, 2002  
MEDIUM: print  
CONFERENCE/MEETING: Annual Meeting of Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002  
ISSN: 0892-6638  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: LF-B25 is an epithelial mitogen and a member of the FGF growth factor family. The purpose of these studies was to evaluate LF-B25-induced proliferation of pulmonary epithelial cells in rats and monkeys when administered intratracheally (IT). Rats receiving an IT dose of LF-B25 were injected with BrdU 24 hours later, and BrdU+ alveolar cells quantified. An IT infusion at a dose of 0.1, 0.3 or 1 mg/kg induced a significant 10-fold increase in the number of BrdU+ cells per microscopic field compared to controls. Histologic evaluation of H&E stained sections revealed no fibrosis, but showed alveolar hyperplasia characterized by the "knobby proliferation" associated with Type II pneumocyte cell division. In the monkey study, following administration of LF-B25 (1 mg/kg, IT), the number of BrdU positive alveolar cells was significantly increased over controls (65 BrdU+ cells/field vs 4). In addition, bronchial epithelial cells exhibited a robust proliferative response following infusion of LF-B25. Because of its specific proliferative effect on airway and alveolar epithelial cells, LF-B25 is being considered as a clinical candidate for the treatment of lung diseases such as ARDS, IPF, asthma and COPD.

2/7/6 (Item 6 from file: 5)  
DIALOG(R)File 5: Biosis Previews(R)  
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13211095 BIOSIS NO.: 200100418244  
Granulocyte-macrophage-colony-stimulating-factor (GM-CSF) expression in the human lung: An immunohistochemical analysis.  
AUTHOR: Burg J(a); Bittinger F(a); Krump-Konvalinkova V(a); Kirkpatrick C J (a)  
AUTHOR ADDRESS: (a) Institute of Pathology, Johannes Gutenberg University of Mainz, Mainz\*\*Germany

JOURNAL: Pathology Research and Practice 197 (5):p334 2001  
MEDIUM: print  
CONFERENCE/MEETING: 85th Meeting of the German Society of Pathology  
Muenster, Germany June 06-09, 2001  
ISSN: 0344-0338  
RECORD TYPE: Citation  
LANGUAGE: English  
SUMMARY LANGUAGE: English

2/7/7 (Item 7 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
(c) 2003 BIOSIS. All rts. reserv.

12709828 BIOSIS NO.: 200000463330  
Apoptosis in lung pathophysiology.  
AUTHOR: Fine Alan; Janssen-Heininger Yvonne; Soultanakis Rebecca P; Swisher  
Stephen G; Uhal Bruce D(a)  
AUTHOR ADDRESS: (a)Dept. of Physiology, Michigan State Univ., 310 Giltner  
Hall, East Lansing, MI, 48824\*\*USA  
JOURNAL: American Journal of Physiology 279 (3 Part 1 of 2):pL423-L427  
September, 2000  
MEDIUM: print  
ISSN: 0002-9513  
RECORD TYPE: Abstract  
LANGUAGE: English  
SUMMARY LANGUAGE: English

ABSTRACT: As recently as 1993, fewer than 10 manuscripts had been published on the topic of apoptosis specifically in the lung. Although that number is increasing, far fewer papers appear each year on apoptosis in the lung than in the other major organs. Therefore, our knowledge of this important aspect of lung cell physiology is relatively rudimentary. Recent literature is beginning to define important roles for apoptosis in normal lung cell turn-over, lung development, and the pathogenesis of diseases such as **interstitial pulmonary fibrosis**, acute respiratory distress syndrome, and chronic obstructive pulmonary disease. Although the involvement of lung cell apoptosis in each of these examples seems clear, the many factors comprising the normal and abnormal regulation of cell death remain to be elucidated and are likely to be different in each situation. The definition of those factors will be an exciting and challenging field of research for many years to come. In that context, the goal of this symposium was to discuss, from a physiological perspective, some of the most recent and exciting advances in the definition of signaling mechanisms involved in the regulation of apoptosis specifically in lung cell populations.

2/7/8 (Item 8 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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11788689 BIOSIS NO.: 199900034798  
Surfactant proteins A and D: Disease markers.  
AUTHOR: Kuroki Yoshio(a); Takahashi Hiroki; Chiba Hirofumi; Akino Toyooki  
AUTHOR ADDRESS: (a)Dep. Biochem., Sapporo Med. Univ. Sch. Med., South-1  
West-17, Chuo-ku, Sapporo 060-8556\*\*Japan  
JOURNAL: Biochimica et Biophysica Acta 1408 (2-3):p334-345 Nov. 19, 1998  
ISSN: 0006-3002  
DOCUMENT TYPE: Literature Review  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: The abundant and restricted expression of surfactant proteins

SP-A and SP-D within the lung makes these collectins specific markers for lung diseases. The measurement of SP-A and SP-D in amniotic fluids and tracheal aspirates reflects lung maturity and the production level of the lung surfactant in infants with respiratory distress syndrome (RDS). The SP-A concentrations in bronchoalveolar lavage (BAL) fluids are significantly decreased in patients with acute respiratory distress syndrome (ARDS) and also in patients at risk to develop ARDS. The prominent increase of these proteins in BAL fluids and sputum is diagnostic for pulmonary alveolar proteinosis (PAP). The concentrations of SP-A and SP-D in BAL fluids from patients with idiopathic pulmonary fibrosis (IPF) and interstitial pneumonia with collagen vascular diseases (IPCD) are rather lower than those in healthy controls and the SP-A/phospholipid ratio may be a useful marker of survival prediction. SP-A and SP-D appear in the circulation in specific lung diseases. Their serum concentrations significantly increase in patients with PAP, IPF and IPCD. The successive monitoring of serum levels of SP-A and SP-D may predict the disease activity. The serum SP-A levels increase in patients with ARDS. SP-A is also a marker for lung adenocarcinomas and can be used to differentiate lung adenocarcinomas from other types and metastatic cancers from other origins, and to detect metastasis of lung adenocarcinomas.

2/7/9 (Item 9 from file: 5)  
DIALOG(R)File 5:BIOSIS Previews(R)  
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11658919 BIOSIS NO.: 199800440650  
Elevated transforming growth factor-alpha levels in bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome.  
AUTHOR: Maddes David K(a); Rubenfeld Gordon; Klima Lawrence D; Milberg John A; Steinberg Kenneth P; Martin Thomas R; Raghu Ganesh; Hudson Leonard D; Clark Joan G  
AUTHOR ADDRESS: (a)Fred Hutchinson Cancer Res. Center, 1124 Columbia St. M677, Seattle, WA 98104-2092\*\*USA  
JOURNAL: American Journal of Respiratory and Critical Care Medicine 158 (2):p424-430 Aug., 1998  
ISSN: 1073-449X  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: The acute respiratory distress syndrome (ARDS) frequently results in a fibroproliferative response that precludes effective alveolar repair. Transforming growth factor-alpha (TGF-alpha), a potent epithelial and mesenchymal cell mitogen, may modulate the response to lung injury. In this study, we determined whether bronchoalveolar lavage fluid (BALF) concentrations of TGF-alpha are increased during the first 2 wk after the onset of ARDS and, if so, whether increased TGF-alpha levels in lavage fluid are associated with increased levels of procollagen peptide III (PCP III), a biological marker of fibroproliferation, and with increased fatality rates. We enrolled 74 consecutive patients with ARDS prospectively identified on admission to the intensive care unit of a tertiary care hospital, and 11 patients with chronic interstitial lung disease. Thirteen healthy volunteers served as control subjects. TGF-alpha-concentrations were measured in BALF recovered on Days 3, 7, and 14 after the onset of ARDS (total of 130 lavage samples). TGF-alpha was detected in the lavage fluid of 90% of patients with ARDS (67 of 74), and in 100% of patients with idiopathic pulmonary fibrosis (IPF) (10 of 10), but in none of 13 normal volunteers. At each day tested, the median lavage TGF-alpha level of patients with ARDS was significantly higher than that of normals. The overall fatality rate was 45% (33 of 74 patients). In a univariate analysis, the median TGF-alpha levels in

nonsurvivors were 1.5-fold higher at Day 7 ( $p = 0.06$ ) and 1.8-fold higher at Day 14 ( $p = 0.048$ ). The fatality rate was 4 times higher (CI 1.6, 17.5) for patients with both increased lavage TGF-alpha and PCP III concentrations at Day 7 than for patients with low TGF-alpha and PCP III values, indicating a synergistic relationship between TGF-alpha and PCP III. We conclude that increased levels of TGF-alpha in BALF are common in patients with ARDS and that lavage TGF-alpha is associated with a marker of the fibroproliferative response in sustained ARDS.

2/7/10 (Item 10 from file: 5)  
DIALOG(R)File 5: Biosis Previews(R)  
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11480952 BIOSIS NO.: 199800262284  
Characterization of N-acetylcysteine and ambroxol in anti-oxidant therapy.  
AUTHOR: Gillissen A(a); Nowak D  
AUTHOR ADDRESS: (a)Univ. Hosp. Bonn, Dep. Internal Med., Div. Pneumol.,  
Sigmund-Freud-Str. 25, D-53105 Bonn\*\*Germany  
JOURNAL: Respiratory Medicine 92 (4):p609-623 April, 1998  
ISSN: 0954-6111  
DOCUMENT TYPE: Literature Review  
RECORD TYPE: Abstract  
LANGUAGE: English

**ABSTRACT:** Reactive free oxygen radicals are known to play an important role in the pathogenesis of various lung diseases such as idiopathic pulmonary fibrosis (IPF), adult respiratory distress syndrome (ARDS) or cystic fibrosis (CF). They can originate from endogenous processes or can be part of exogenous exposures (e.g. ozone, cigarette smoke, asbestos fibres). Consequently, therapeutic enhancement of anti-oxidant defence mechanisms in these lung disorders seems a rational approach. In this regard, N-acetyl-L-cysteine (NAC) and ambroxol have both been frequently investigated. Because of its SH group, NAC scavenges  $H_2O_2$  (hydrogen peroxide),  $\cdot OH$  (hydroxyl radical), and  $HOCl$  (hypochlorous acid). Furthermore, NAC can easily be deacetylated to cysteine, an important precursor of cellular glutathione synthesis, and thus stimulate the cellular glutathione system. This is most evident in pulmonary diseases characterized by low glutathione levels and high oxidant production by inflammatory cells (e.g. in IPF and ARDS). NAC is an effective drug in the treatment of paracetamol intoxication and may even be protective against side-effects of mutagenic agents. In addition NAC reduces cellular production of pro-inflammatory mediators (e.g. TNF-alpha, IL-1). Also, ambroxol (trans-4-(2-amino-3,5-dibromobenzylamino)-cyclohexane hydrochloride) scavenges oxidants (e.g.  $\cdot OH$ ,  $HOCl$ ). Moreover, ambroxol reduces bronchial hyperreactivity, and it is known to stimulate cellular surfactant production. In addition, ambroxol has anti-inflammatory properties owing to its inhibitory effect on the production of cellular cytokines and arachidonic acid metabolites. For both substances effective anti-oxidant and antiinflammatory function has been validated when used in micromolar concentrations. These levels are attainable in vivo in humans. This paper gives an up-to-date overview about the current knowledge of the hypothesis that oxidant-induced cellular damage underlies the pathogenesis of many human pulmonary diseases, and it discusses the feasibility of anti-oxidant augmentation therapy to the lung by using NAC or ambroxol.

2/7/11 (Item 11 from file: 5)  
DIALOG(R)File 5: Biosis Previews(R)  
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11385474 BIOSIS NO.: 199800166806

Soluble complement receptor type 1 (CD35) in bronchoalveolar lavage of inflammatory lung diseases.

AUTHOR: Hamacher J; Sadallah S; Schifferli J A; Villard J; Nicod L P(a)

AUTHOR ADDRESS: (a)Pulmonary Div., Hopital Cantonal Univ., CH-1211 Geneva 14\*\*Switzerland

JOURNAL: European Respiratory Journal 11 (1):p112-119 Jan., 1998

ISSN: 0903-1936

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

**ABSTRACT:** Complement receptor type 1 (CR1) (CD35; C3b/C4b receptor) is a transmembrane protein of many haematopoietic cells. Once cleaved, soluble complement receptor type 1 (sCR1) exerts opposite effects as a powerful inhibitor of complement. This study addressed both the question of whether sCR1 was found in bronchoalveolar lavage (BAL) of normals and patients with various inflammatory disease, and its possible origin. In this retrospective study covering specimen and clinical data of 124 patients with acute and chronic inflammatory lung pathologies, BAL supernatants were analysed by enzyme-linked immunosorbent assay technique for sCR1. Correlations were made between the sCR1 levels obtained and the constituents of BAL. Human alveolar macrophages were cultivated in order to determine their secretory capacity of sCR1. Alveolar macrophages from normal subjects were shown to release sCR1 in vitro. In addition, sCR1 was present in BAL of normal controls and was significantly increased in acute inflammatory lung diseases such as acute respiratory distress syndrome (ARDS), bacterial and Pneumocystis carinii pneumonia, as well as in chronic inflammatory diseases such as **interstitial lung fibrosis** and sarcoidosis. In BAL of **ARDS**, bacterial, and P. carinii pneumonia, there was a good correlation between sCR1 and the absolute neutrophil counts. In sarcoidosis, a correlation was found with BAL lymphocyte counts. Serum sCR1 was not increased in patients compared to controls. Soluble complement receptor type 1 (sCR1) is found in the bronchoalveolar lavage in health as well as in acute and chronic inflammatory disease. Alveolar macrophages are capable of releasing sCR1 in vitro and may be the main physiological source of sCR1 in the alveoli. The good correlation between sCR1 and the absolute neutrophil or lymphocyte numbers in bronchoalveolar lavage of inflammatory diseases suggests a predominant role of leucocytes for the release of sCR1 in such conditions. The release of this inhibitor of complement may be crucial to control and reduce complement activation and thus prevent lung injury.

2/7/12 (Item 12 from file: 5)

DIALOG(R)File 5: Biosis Previews(R)

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10750778 BIOSIS NO.: 199799371923

Proteoglycan deposition in pulmonary fibrosis.

AUTHOR: Bensadoun Eric S; Burke Adrian K; Hogg James C; Roberts Clive R(a)

AUTHOR ADDRESS: (a)Univ. British Columbia Pulmonary Res. Lab., Dep. Med., Univ. British Columbia, St. Paul's Hosp.,\*\*Canada

JOURNAL: American Journal of Respiratory and Critical Care Medicine 154 (6 PART 1):p1819-1828 1996

ISSN: 1073-449X

RECORD TYPE: Abstract

LANGUAGE: English

**ABSTRACT:** This study compares the deposition of proteoglycans in the extracellular matrix of the lung lesions of the **adult respiratory** distress syndrome (ARDS) and bronchiolitis obliterans organizing pneumonia (BOOP) to those present in idiopathic pulmonary fibrosis (IPF). Tissue from individuals with **ARDS**

(n = 7), BOOP (n = 5), **IPF** (n = 5), and control subjects (n = 5) was examined for glycosaminoglycans and collagen by histochemistry, and for hyaluronan; versican, decorin, biglycan, Types I and III collagen, type I procollagen and alpha-smooth muscle actin (alpha-SMA) using immunohistochemistry. The results showed that glycosaminoglycan deposition in the lesions of **ARDS**, BOOP, and **IPF** corresponded to the deposition of versican. Versican localized to the thickened interstitium in the fibroproliferative phase of **ARDS**, to the intraluminal buds in BOOP, and to early fibroblast foci in **IPF**. The versican-rich areas contained little mature collagen, but the myofibroblasts in these areas stained for type I procollagen, suggesting early collagen synthesis, and stained intracellularly for decorin. The localization of versican in **ARDS**, BOOP, and **IPF** suggests that this proteoglycan may influence early repair processes in the lung.

2/7/13 (Item 13 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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10522170 BIOSIS NO.: 199699143315

Heme oxygenase-1: Function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury.

AUTHOR: Choi Augustine M K(a); Alam Jawed

AUTHOR ADDRESS: (a)Div. Pulmonary and Critical Care, Johns Hopkins Sch. Med., Ross Research Bldg., Room 858, 720 Ru\*\*USA

JOURNAL: American Journal of Respiratory Cell and Molecular Biology 15 (1):p9-19 1996

ISSN: 1044-1549

DOCUMENT TYPE: Literature Review

RECORD TYPE: Abstract

LANGUAGE: English

**ABSTRACT:** Accumulating evidence suggests that oxidative stress plays a central role in the pathogenesis of many pulmonary diseases including **adult respiratory distress syndrome**, emphysema, asthma, bronchopulmonary dysplasia, and **interstitial pulmonary fibrosis**. The morbidity and mortality of these diseases remain high even with optimal medical management. In our attempts to devise new therapies for these disorders, it is crucial to improve our understanding of the basic mechanism(s) of oxidant-induced lung injury. A major line of investigation seeks to characterize the cellular and molecular responses of the lung to oxidant insults. Much progress has been made in our understanding of the role of the "classic" antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase) in mediating the lung's resistance against oxidant lung injury. However, it is becoming clear that other oxidant-induced gene products may also play vital roles in the lung's adaptive and/or protective response to oxidative stress. One such stress-response protein is heme oxygenase-1, HO-1. Since the identification of HO-1 in 1968, many of the studies involving this enzyme were understandably focused on the regulation and function of HO-1 in heme metabolism. This emphasis is self-evident as HO-1 catalyzes the first and rate-limiting step in heme degradation. Interestingly, however, evidence accumulated over the past 25 years demonstrates that HO-1 is induced not only by the substrate heme but also by a variety of non-heme inducers such as heavy metals, endotoxin, heat shock, inflammatory cytokines, and prostaglandins. The chemical diversity of HO-1 inducers led to the speculation that HO-1, besides its role in heme degradation, may also play a vital function in maintaining cellular homeostasis. Further support for this hypothesis was provided by Tyrrell and colleagues who showed in 1989 that HO-1 is also highly induced by a variety of agents causing oxidative stress. Subsequently, many investigators have focused their attention on the function and regulation of HO-1 in various in vitro and in vivo models of oxidant-mediated

cellular and tissue injury. The magnitude of HO-1 induction after oxidative stress and the wide distribution of this enzyme in systemic tissues coupled with the intriguing biological activities of the catalytic byproducts, carbon monoxide, iron, and bilirubin, makes HO-1 a highly attractive and interesting candidate stress-response protein which may play key role(s) in mediating protection against oxidant-mediated lung injury. This review will focus on the current understanding of the physiological significance of HO-1 induction and the molecular regulation of HO-1 gene expression in response to oxidative stress. We hope that this discussion will stimulate interest and investigations into a field which is still largely uncharted in the pulmonary research community.

2/7/14 (Item 14 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
(c) 2003 BIOSIS. All rts. reserv.

09912855 BIOSIS NO.: 199598367773  
Soluble form of P-selectin in plasma is elevated in acute lung injury.  
AUTHOR: Sakamaki Fumio; Ishizaka Akitoshi(a); Handa Makoto; Fujishima Seitaro; Urano Tetsuya; Sayama Koichi; Nakamura Hidetoshi; Kanazawa Minoru; Kawashiro Takeo; Katayama Masahiko; Ikeda Yasuo  
AUTHOR ADDRESS: (a)Dep. Med., Sch. Med., Keio Univ., 35 Shinanomachi, Shinjuku-ku, Tokyo 160\*\*Japan  
JOURNAL: American Journal of Respiratory and Critical Care Medicine 151 (6):p1821-1826 1995  
ISSN: 1073-449X  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: A number of adhesion molecules on neutrophils and the pulmonary capillary endothelium mediate the neutrophil accumulation in the lungs at the onset of **adult respiratory distress syndrome** or acute lung injury (ALI). P-selectin, located on both vascular endothelial cells and platelets, has been shown to be one of these neutrophil-endothelial cell adhesion molecules. In this study, we measured the soluble form of P-selectin in plasma (PPS) from 19 patients (surviving, 11; deceased, 8) with ALI due to various causes and assessed the clinical significance of this measurement. Twelve healthy subjects and 29 patients with other pulmonary diseases, including idiopathic pulmonary fibrosis (IPF) (n = 8), sarcoidosis (n = 5), pneumonia (n = 8), and sepsis without ALI (n = 8) were also studied for comparison. PPS in patients with ALI (474.5 +- 366.8 ng/ml, mean +- SD) were significantly higher than those in control subjects (98.8 +- 39.7, p lt 0.01) and in patients with IPF (210.4 +- 76.6, p lt 0.05), sarcoidosis (135.2 +- 71.5, p lt 0.05), pneumonia (225.3 +- 81.0, p lt 0.05), and sepsis without ALI (271.8 +- 46.5, p lt 0.05). There was no significant difference in PPS levels between seven patients with and 12 patients without multiple organ failure. Lung injury scores correlated significantly with the PPS level (r = 0.605, p lt 0.05). PPS levels of deceased patients with ALI (841.0 +- 252.4) were significantly higher than those of surviving patients with ALI (208.0 +- 109.2, p lt 0.01). These findings suggest that PPS levels were elevated in the plasma of patients with ALI, especially in those who subsequently died, as compared with those in patients with other pulmonary disease or sepsis without ALI.

2/7/15 (Item 15 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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09238501 BIOSIS NO.: 199497246871  
Serum concentration of 7S collagen and prognosis in patients with the



**adult respiratory** distress syndrome.

AUTHOR: Kawamura Masafumi(a); Yamasawa Fumihiko; Ishizaka Akitoshi; Kato Ryoichi; Kikuchi Koji; Kobayashi Koichi; Aoki Takuya; Sakamaki Fumio; Hasegawa Naoki; Kawashiro Takeo; Ishihara Tsuneo

AUTHOR ADDRESS: (a)Dep. Surgery, Sch. Med., Keio Univ., 35 Shinanomachi, Shinjuku-ku, Tokyo 160\*\*Japan

JOURNAL: Thorax 49 (2):p144-146 1994

ISSN: 0040-6376

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

**ABSTRACT:** Background - 7 S collagen, an N-terminal peptide of type IV collagen, is a primary constituent of the basement membrane. To evaluate whether the serum concentration of 7S collagen reflects the severity of inflammatory lung disease, the serum concentration of 7S collagen was measured in patients with **adult respiratory** distress syndrome (**ARDS**) and idiopathic pulmonary fibrosis (**IPF**). Methods - A radioimmunoassay was used for the measurement of 7S collagen. Gas exchange abnormality was expressed as the arterial oxygen tension (PaO-2) divided by the fractional concentration of inspired oxygen (FiO-2). Results - The mean (SD) concentration of 7S collagen was 2.7 (0.9) ng/ml in 10 healthy subjects, 5.0 (1.5) ng/ml in 11 patients with **IPF**, and 14.8 (9.7) ng/ml in 13 patients with **ARDS**. Significant differences were observed between the patients with **ARDS** and both healthy subjects and the patients with **IPF**. In the patients with **ARDS** serum concentrations of 7S collagen were strongly related to PaO-2/FiO-2 ( $r = -0.61$ ). Moreover, the mean (SD) serum concentration of 7S collagen in the eight patients with **ARDS** who died (19.5 (10.2) ng/ml) was considerably higher than that of the five who survived (7.1 (2.1) ng/ml). Conclusion - These results suggest that serum levels of the 7S fragment of type IV collagen may have some prognostic value in **ARDS**.

2/7/16 (Item 16 from file: 5)

DIALOG(R)File 5:BIOSIS Previews(R)

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08767014 BIOSIS NO.: 199395056365

Correlative study of **adult respiratory** distress syndrome by light, scanning, and transmission electron microscopy.

AUTHOR: Anderson W Robert(a); Thielen Kimberlee

AUTHOR ADDRESS: (a)Dep. Pathol., Hennepin County Med. Cent., 701 Park Ave. South, Minneapolis, Minn. 55415

JOURNAL: Ultrastructural Pathology 16 (6):p615-628 1992

ISSN: 0191-3123

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

**ABSTRACT:** The sequential pulmonary changes occurring in the evolution of **adult respiratory** distress syndrome (**ARDS**) were studied in 35 patients by correlative light, scanning, and transmission electron microscopy. The causes of **ARDS** were diverse, the major ones being sepsis or aspiration. Patient survival ranged from 3 to 51 days. The acute stage in patients surviving 2 to 7 days was characterized by an exudative reaction with a predominance of hyaline membranes. This acute stage merged with and was replaced by a subacute reparative stage in patients surviving 7 to 14 days, which in turn was replaced by a chronic fibroproliferative stage complicated by **interstitial pulmonary fibrosis** and a deranged acinar architecture. Correlation of findings by scanning electron microscopy with those by light and transmission electron microscopy provided an added dimension to

understanding of the evolving stages of **ARDS** and demonstrated that type 2 pneumocytes contributed to the fibroproliferative stage through organization of hyaline membranes and re-epithelialization of alveoli.

2/7/17 (Item 17 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
(c) 2003 BIOSIS. All rts. reserv.

08307091 BIOSIS NO.: 000094069414  
FIBRONECTIN A VERSATILE MATRIX PROTEIN WITH ROLES IN THORACIC DEVELOPMENT  
REPAIR AND INFECTION  
AUTHOR: LIMPER A H; ROMAN J  
AUTHOR ADDRESS: THORACIC DISEASES, MAYO CLINIC, ROCHESTER, MINN. 55905.  
JOURNAL: CHEST 101 (6). 1992. 1663-1673. 1992  
FULL JOURNAL NAME: Chest  
CODEN: CHETB  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

ABSTRACT: Fibronectin, a dimeric cell-adhesive extracellular matrix glycoprotein, is secreted by mesenchymal cells and assembled into insoluble matrices which have important biological functions in embryologic development as well as in tissue response to injury. Fibronectin interacts with numerous cell types including mesenchymal cells and inflammatory cells which bear appropriate fibronectin receptors. In vitro, fibronectin serves as an adhesive substrate and promotes cell proliferation and cytodifferentiation. During development, fibronectin-rich matrices are deposited in specific location and regulate the directional migration of embryonic cells. In particular, fibronectin matrices appear to be of critical importance to normal cardiopulmonary development. Following embryologic development, the tissue expression of fibronectin is greatly reduced, but increases markedly following tissue injury, where newly expressed fibronectin matrices appear critical to tissue repair. Recent evidence has documented increased expression of fibronectin in numerous pulmonary conditions including the **adult respiratory distress syndrome (ARDS)**, bronchiolitis obliterans organizing pneumonia (BOOP) and idiopathic pulmonary fibrosis (IPF). Additionally, fibronectin also interacts with a large number of microorganisms and therefore also is potentially important in microbial adherence to airway epithelium and subsequent infections of the respiratory system.

2/7/18 (Item 18 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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06722762 BIOSIS NO.: 000088032188  
HISTOCHEMICAL EVALUATION OF LUNG COLLAGEN CONTENT IN ACUTE AND CHRONIC INTERSTITIAL DISEASES  
AUTHOR: SALDIVA P H N; DELMONTE V C; CARVALHO C R R D; KAIRALLA R A; AULER J O C JR  
AUTHOR ADDRESS: LAB. POLUICAO ATOMSFERICA EXP., DEP. PATHOL., FAC. MED. USP, AV DR ARNALDO 455, SAO PAULO, BRAZIL 01246.  
JOURNAL: CHEST 95 (5). 1989. 953-957. 1989  
FULL JOURNAL NAME: Chest  
CODEN: CHETB  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

ABSTRACT: The collagen and its aggregational state was histochemically measured in interstitial lung diseases. Open chest biopsies of ten patients with **adult respiratory distress syndrome**, seven

patients with sarcoidosis, and nine patients with fibrosis associated with connective tissue diseases and with idiopathic pulmonary fibrosis (IPF/CTD) were compared with eight samples of normal lungs. The collagen content of diseased lungs was significantly increased when compared to control lungs, but no difference was observed among the pathologic groups. The analysis of collagen aggregational state showed maximal aggregation in IPF/CTD, followed by sarcoidosis, ARDS, and control lungs, in decreasing order. The results suggest that measurement of collagen aggregation coupled with collagen content could be used in the evaluation of interstitial lung disease and encourage the use of new techniques in order to better explain the dramatic histologic and functional alterations observed in many disease-associated lung processes.

2/7/19 (Item 19 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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04686760 BIOSIS NO.: 000079099889  
EXTRACELLULAR MATRIX IN NORMAL AND FIBROTIC HUMAN LUNGS  
AUTHOR: RAGHU G; STRIKER L J; HUDSON L D; STRIKER G E  
AUTHOR ADDRESS: DIVISION OF RESPIRATORY DISEASES AND CRITICAL CARE  
MEDICINE, DEPARTMENT OF MEDICINE, RM-12, UNIVERSITY OF WASHINGTON,  
SEATTLE, WASHINGTON 98195.  
JOURNAL: AM REV RESPIR DIS 131 (2). 1985. 281-289. 1985  
FULL JOURNAL NAME: American Review of Respiratory Disease  
CODEN: ARDSB  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

ABSTRACT: Polyclonal affinity-purified antibodies to human collagen types I, III and IV, and laminin were used to compare the extracellular matrix (ECM) in 10 normal and 32 abnormal lungs by indirect immunofluorescence. In normal lungs, type IV collagen and laminin codistributed in a uniform linear pattern along the epithelial and endothelial basement membranes. Type III collagen was found with the alveolar septa and interstitium in an interrupted ribbonlike pattern and was aggregated at the entrance rings of the alveoli. Type I collagen was distributed irregularly within the alveolar wall and was less prominent than type III collagen. In patients with pulmonary disease not characterized by interstitial fibrosis (n = 15), the distribution of ECM components studied was essentially normal. In pulmonary disease in which interstitial fibrosis was the characteristic feature, such as idiopathic pulmonary fibrosis (IPF) and adult respiratory distress syndrome (ARDS) (n = 17), collagen types I and III accumulated in the expanded interstitium. Type III collagen was initially predominant in the thickened alveolar septa and interstitium, whereas type I collagen appeared to be the principal collagen at later stages in the disease course. The basement membrane was disrupted early in the disease course with invasion of the alveolar spaces by interstitial collagens similar in type to those present in the adjacent interstitium.

2/7/20 (Item 20 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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03808809 BIOSIS NO.: 000025061882  
ADULT RESPIRATORY DISTRESS SYNDROME A REVIEW  
AUTHOR: HASLETON P S  
AUTHOR ADDRESS: DEP. PATHOLOGY, WYTHENSHAW HOSP., SOUTHMOOR RD.,  
MANCHESTER M23 9LT.  
JOURNAL: HISTOPATHOLOGY (OXF) 7 (3). 1983. 307-332. 1983

FULL JOURNAL NAME: HISTOPATHOLOGY (Oxford)  
CODEN: HISTD  
DOCUMENT TYPE: Review  
RECORD TYPE: Citation  
LANGUAGE: ENGLISH

2/7/21 (Item 21 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
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02082713 BIOSIS NO.: 000013102831  
TOTAL LUNG COLLAGEN CONTENT RAPIDLY INCREASES DURING ADULT  
RESPIRATORY DISTRESS SYNDROME  
AUTHOR: ZAPOL W M; TRELSTAD R; COFFEY J; TSAI I; SALVADOR R  
JOURNAL: AM REV RESPIR DIS 115 (4 PART 2). 1977 183 1977  
FULL JOURNAL NAME: American Review of Respiratory Disease  
CODEN: ARDSB  
DOCUMENT TYPE: Meeting  
RECORD TYPE: Citation

2/7/22 (Item 1 from file: 73)  
DIALOG(R)File 73:EMBASE  
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11370705 EMBASE No: 2001385817  
High-resolution CT of idiopathic interstitial pneumonias  
Lynch D.A.  
Dr. D.A. Lynch, Department of Radiology, Box A030, Univ. of Colorado  
Hlth. Sci. Center, 4200 East Ninth Avenue, Denver, CO 80262 United  
States  
Radiologic Clinics of North America ( RADIOL. CLIN. NORTH AM. ) (United  
States) 2001, 39/6 (1153-1170)  
CODEN: RCNAA ISSN: 0033-8389  
DOCUMENT TYPE: Journal ; Review  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH  
NUMBER OF REFERENCES: 70

Important recent changes have occurred in our understanding of the IIPs.  
**IPF** (characterized histologically as UIP) is recognized as a  
progressive disease with a relatively poor prognosis, and with a  
characteristic CT appearance. The radiologist must be able to distinguish  
between UIP and the other IIPs. Complications of **IPF** include  
accelerated progression, lung cancer, and secondary infection. NSIP has a  
better prognosis than **IPF**, and has ground-glass attenuation as its  
salient CT feature. COP (formerly known as BOOP) is included as an IIP  
because its clinical, physiologic, and imaging features overlap with those  
of the other IIPs. It is characterized on CT by consolidation and  
ground-glass attenuation. AIP is the idiopathic form of **ARDS**. LIP and  
DIP are less common IIPs, both characterized by ground-glass attenuation.

2/7/23 (Item 2 from file: 73)  
DIALOG(R)File 73:EMBASE  
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10809470 EMBASE No: 2000291121  
Smoking-related interstitial lung diseases  
Nagai S.; Hoshino Y.; Hayashi M.; Ito I.  
Dr. S. Nagai, Dept. of Respiratory Medicine, Graduate School of Medicine,  
Kyoto University, Kyoto 606-8507 Japan  
AUTHOR EMAIL: nagai@kuhp.kyoto-u.ac.jp  
Current Opinion in Pulmonary Medicine ( CURR. OPIN. PULM. MED. ) (United

States) 2000, 6/5 (415-419)  
CODEN: COPMF ISSN: 1070-5287  
DOCUMENT TYPE: Journal; Review  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH  
NUMBER OF REFERENCES: 40

Interstitial lung diseases (ILDs) encompass diverse clinicopathological disease entities ranging from idiopathic interstitial pneumonia (IIP) to interstitial pneumonia accompanied by collagen vascular diseases and diseases related to smoking, drug reactions, occupational environments, infections, and malignancies. Our focus is on the smoking-related interstitial lung diseases. Specifically, we focus on reports suggesting that chronic smoking is deeply involved in the disease pathogenesis and on reports suggesting that chronic smoking may influence the clinical course in terms of either disease severity or progression. Pulmonary Langerhans' cell granulomatosis (PLCG), desquamative interstitial pneumonia (DIP), respiratory bronchiolitis with interstitial lung diseases (RB-ILD), interstitial pneumonia associated with rheumatoid arthritis, acute respiratory distress syndrome (ARDS), and idiopathic pulmonary fibrosis (IPF) are covered. (C) 2000 Lippincott Williams and Wilkins, Inc.

2/7/24 (Item 3 from file: 73)  
DIALOG(R)File 73:EMBASE  
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07156398 EMBASE No: 1998045075

The significance of bronchial dilatation on CT in patients with **adult respiratory distress syndrome**  
Howling S.J.; Evans T.W.; Hansell D.M.  
Dr. D.M. Hansell, Department of Radiology, Royal Brompton Hospital, Sydney Street, London SW3 6NP United Kingdom  
Clinical Radiology (CLIN. RADIOLOG.) (United Kingdom) 1998, 53/2 (105-109)  
CODEN: CLRAA ISSN: 0009-9260  
DOCUMENT TYPE: Journal; Article  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH  
NUMBER OF REFERENCES: 18

Aims: Irreversible bronchial dilatation on computed tomography (CT) is seen in patients with **interstitial pulmonary fibrosis** whereas reversible dilatation of the bronchi is a feature of many conditions. The aim of this study was to determine whether airways dilatation, which is a frequent finding in **adult respiratory distress syndrome (ARDS)**, is reversible and to establish whether persistent airways dilatation is accompanied by other CT signs of established interstitial fibrosis. Method: Thin section CT scans of 16 patients with **ARDS** were analysed at a lobar level in the acute phase of the disease and at follow-up (median interval 6.3 months) for the presence and extent of a ground-glass pattern, reticular or linear opacities and the presence or absence of bronchial dilatation. Results: 95 out of 96 (99%) lobes on the initial CT scan showed ground-glass pattern. Airway dilatation was present in 65 of 95 (68%) of these lobes. On follow-up CT the airways remained dilated in 60 of 65 (92%) lobes and, in the majority of these, 53 of 60 (88%), a reticular and linear pattern with associated distortion had developed. A coexisting ground-glass pattern was present in 12 of 60 (20%) lobes on follow-up CT, but was the sole residual abnormality in only one lobe. Conclusion: In patients with **ARDS**, dilatation of the airways within areas of ground-glass pattern is a frequent observation in the acute phase of the condition and tends to persist at follow-up, usually accompanied by the CT features of supervening pulmonary fibrosis.

2/7/25 (Item 4 from file: 73)  
DIALOG(R)File 73:EMBASE  
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07120407 EMBASE No: 1998006411

Serum-soluble adhesion molecules in patients with idiopathic pulmonary fibrosis and acute respiratory distress syndrome

Ashitani J.-I.; Mukae H.; Ihiboshi H.; Hiratsuka T.; Taniguchi H.; Mashimoto H.; Matsukura S.

J.-I. Ashitani, Third Dept. of Internal Medicine, Miyazaki Medical College, Miyazaki Japan

Japanese Journal of Thoracic Diseases ( JPN. J. THORAC. DIS. ) (Japan) 1997, 35/9 (942-947)

CODEN: NKYZA ISSN: 0301-1542

DOCUMENT TYPE: Journal; Article

LANGUAGE: JAPANESE SUMMARY LANGUAGE: ENGLISH; JAPANESE

NUMBER OF REFERENCES: 20

We measured the concentrations of soluble adhesion molecules in serum obtained from healthy volunteers and from patients with idiopathic pulmonary fibrosis (IPF) and the acute respiratory distress syndrome (ARDS). The concentrations of soluble intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin, and P-selectin were significantly higher in serum from the patients than in serum from the healthy volunteers. In patients with IPF, the concentration of soluble ICAM-1 in serum was inversely related to the vital capacity (expressed as a percent of the predicted value). The concentrations of L-selectin in serum from patients with ARDS were significantly lower than those in serum from healthy volunteers and from patients with IPF. These data suggest that serum-soluble adhesion molecules may be useful as markers of disease activity, severity and prognosis in ARDS and IPF.

2/7/26 (Item 5 from file: 73)  
DIALOG(R)File 73:EMBASE  
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06519657 EMBASE No: 1996185319

The role of cytokines in human lung fibrosis

Vaillant P.; Menard O.; Vignaud J.-M.; Martinet N.; Martinet Y.

Fed. Medico-Chirurgicale Pneumologie, CHU de Nancy-Brabois, Allee du Morvan, 54511 Vandoeuvre-les-Nancy France

Monaldi Archives for Chest Disease ( MONALDI ARCH. CHEST DIS. ) (Italy)

1996, 51/2 (145-152)

CODEN: MACDE ISSN: 1122-0643

DOCUMENT TYPE: Journal; Article

LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Fibrosis is a disorder characterized by a qualitative and quantitative alteration of the deposition of extracellular matrix with accumulation of mesenchymal cells in replacement of normal tissue. The sequence of events leading to fibrosis of an organ involves the subsequent processes of injury with inflammation and disruption of the normal tissue architecture, followed by tissue repair with accumulation of mesenchymal cells in this area. A similar sequence of events occurs in wound healing with formation of normal, limited and transient granulation tissue, while in fibrosis, a maladaptive repair leads to an extensive, exaggerated process with functional impairment. Inflammatory cells (mainly mononuclear phagocytes), platelets, endothelial cells, and type II pneumocytes play a direct and indirect role in tissue injury and repair. The evaluation of several human fibrotic lung diseases, five diffuse (idiopathic pulmonary fibrosis (IPF); adult respiratory distress syndrome (ARDS);

coal workers' pneumoconiosis (CWP); Hermansky-Pudlak syndrome (HPS); systemic sclerosis (SS)) and two focal (tumour stroma in lung cancer; and obliterative bronchiolitis (OB) after lung transplantation), has shown that several cytokines participate in the local injury and inflammatory reaction (interleukin-1 (IL-1), interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), and tumour necrosis factor-alpha (TNF-alpha)), while other cytokines are involved in tissue repair and fibrosis (platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-beta (TGF-beta), and basic-fibroblast growth factor (b-FGF)). A better understanding of the cytokines and cytokine networks involved in lung fibrosis leads to the possibility of new therapeutic approaches.

2/7/27 (Item 6 from file: 73)  
DIALOG(R)File 73:EMBASE  
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04837599 EMBASE No: 1991332335  
GSH rescue by N-acetylcysteine  
Ruffmann R.; Wendel A.  
Inpharzam SA, Cadempino Switzerland  
Klinische Wochenschrift (KLIN. WOCHENSCHR.) (Germany) 1991, 69/18  
(857-862)  
CODEN: KLWOA ISSN: 0023-2173  
DOCUMENT TYPE: Journal; Review  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Reduced glutathione (GSH) is the main intracellular low molecular weight thiol. GSH acts as a nucleophilic scavenger and as an enzyme-catalyzed antioxidant in the event of electrophilic/oxidative tissue injury. Therefore, GSH has a major role as a protector of biological structures and functions. GSH depletion has been recognized as a hazardous condition during paracetamol intoxication. Conversely, GSH rescue, meaning recovery of the protective potential of GSH by early administration of N-acetylcysteine (NAC), has been found to be life-saving. Lack of GSH and electrophilic/oxidative injury have been identified among the causes of the **adult respiratory distress syndrome (ARDS)**, **idiopathic pulmonary fibrosis (IPF)**, and the **acquired immunodeficiency syndrome (AIDS)**. Experimental and early clinical data (in **ARDS**) point to the role of NAC in the treatment of these conditions. Recently, orally given NAC has been shown to enhance the levels of GSH in the liver, in plasma, and notably in the bronchoalveolar lavage fluid. Rescue of GSH through NAC needs to be appreciated as an independent treatment modality for an array of different diseases, all of which have one feature in common: pathogenetically relevant loss of GSH.

2/7/28 (Item 7 from file: 73)  
DIALOG(R)File 73:EMBASE  
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04256353 EMBASE No: 1990138896  
Pulmonary fibrosis  
Dunnill M.S.  
Department of Histopathology, John Radcliffe Hospital, Oxford OX3 9DU  
United Kingdom  
Histopathology (HISTOPATHOLOGY) (United Kingdom) 1990, 16/4 (321-329)  
CODEN: HISTD ISSN: 0309-0167  
DOCUMENT TYPE: Journal; Review  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

**Diffuse interstitial pulmonary fibrosis** is the end result of alveolar damage which may occur as a sudden acute incident or as a slowly developing process. Potentiating factors include release of

enzymes and generation of oxygen radicals by granulocytes. Evidence from studies on broncho-alveolar lavage fluid and from immunocyto-chemistry indicate that an autoimmune process has an important but, as yet, not clearly defined role in initiating the disease. The fibrosis is probably due to proliferation of small clones of fast growing fibroblasts of a specific phenotype. Bronchiolitis obliterans, organizing pneumonia, idiopathic pulmonary fibrosis, usual interstitial pneumonia and desquamative interstitial pneumonia represent different aspects of the same condition. Their varied morphological appearances probably reflect the fact that tissue has been taken at different stages in the development of the disease.

2/7/29 (Item 8 from file: 73)  
DIALOG(R)File 73:EMBASE  
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03820259 EMBASE No: 1988269699  
High-frequency jet ventilation in the treatment of idiopathic pulmonary fibrosis complicating ARDS: A nursing challenge  
Smith S.  
Trauma Service, Nursing Lehigh Valley Hospital Center, Allentown, PA  
United States  
Critical Care Nursing Quarterly ( CRIT. CARE NURS. Q. ) (United States)  
1988, 11/3 (29-35)  
CODEN: CCNQE ISSN: 0160-2551  
DOCUMENT TYPE: Journal  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

IPF in ARDS treated with HFJV is an example of one of the numerous disease processes and changing technologies that confront critical care nurses. Nurses must understand the pathophysiology behind the disease process and the mechanics of HFJV to adequately assess, plan, implement, and evaluate the effectiveness of the care that they deliver.

2/7/30 (Item 9 from file: 73)  
DIALOG(R)File 73:EMBASE  
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02647657 EMBASE No: 1984166615  
Interactions of granulocytes with the lungs  
Brigham K.L.; Meyrick B.  
Pulmonary Circulation Center, Department of Medicine, Vanderbilt  
University School of Medicine, Nashville, TN United States  
Circulation Research ( CIRC. RES. ) (United States) 1984, 54/6 (623-635)  
CODEN: CIRUA  
DOCUMENT TYPE: Journal  
LANGUAGE: ENGLISH

Under normal conditions, there is a sizeable pool of marginated granulocytes in the lung circulation which is in dynamic equilibrium with the circulating granulocyte pool. The number of granulocytes in the lungs' microcirculation may depend on pulmonary blood flow or biochemical interactions between granulocytes and pulmonary vascular endothelium, or both. There is some evidence that normal lung function may be affected by granulocytes. Several acute and chronic diseases may result, at least in part, from interactions of granulocytes with the lungs. Acute diffuse lung injury (**adult respiratory** distress syndrome) is characterized by diffuse pulmonary inflammation, and, in animal models, some of the lung dysfunction depends on the presence of granulocytes. Bronchoconstriction and airway hyperreactivity, characteristic of asthma, may be influenced by granulocyte-generated products of arachidonic acid. Granulocyte-derived proteases and oxidants may contribute to the pathogenesis of pulmonary



emphysema and may affect connective tissue synthesis in **interstitial pulmonary fibrosis**. There is some evidence suggesting a connection between granulocytes and chronic pulmonary hypertension. The fact that some interventions which cause pulmonary leukostasis do not cause severe, persistent lung injury indicates that as yet unknown factors may determine whether interactions of granulocytes with the lungs are benign or pathological. Such factors could include the generation of humoral substances, and metabolites of arachidonic acid are particularly interesting in this regard. Research related to interactions of granulocytes with the lungs suggests strongly that such interactions are integral to the pathogenesis of several lung diseases. Understanding those diseases will require further basic studies of granulocyte behavior and the modes of communication between cells intrinsic to the lung and granulocytes.

2/7/31 (Item 1 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
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15074455 22735455 PMID: 12851645  
Significant Involvement of CCL2 (MCP-1) in Inflammatory Disorders of the Lung.

Rose C Edward; Sung Sung-Sang J; Fu Shu Man  
Division of Pulmonary and Critical Care Medicine and the Division of Rheumatology and Immunology, University of Virginia School of Medicine, Charlottesville, VA, USA.

Microcirculation (New York, N.Y. - 1994) (United States) Jul 2003, 10 (3-4) p273-88, ISSN 1073-9688 Journal Code: 9434935

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: In Process

Mounting evidence suggests that CCL2 (MCP-1) and its hematopoietic cell receptor CC chemokine receptor 2 (CCR2) are involved in inflammatory disorders of the lung. In animal models of allergic asthma, idiopathic pulmonary fibrosis (IPF), and bronchiolitis obliterans syndrome (BOS), CCL2 expression and protein production are increased and the disease process is attenuated by CCL2 immunoneutralization. Mechanisms by which CCL2 may be acting include recruitment of regulatory and effector leukocytes; stimulation of histamine or leukotriene release from mast cells or basophils; induction of fibroblast production of transforming growth factor-beta (TGF-beta) and procollagen; and enhancement of Th2 polarization. Recently, polymorphism for CCL2 has been described with increased cytokine-induced release of CCL2 by monocytes and increased risk of allergic asthma. These studies identify potentially important roles for CCL2 in these lung inflammatory disorders. While CCL2 inhibition in patients with acute respiratory distress syndrome (ARDS) may be hazardous by interfering with defense against bacteremia, future studies are needed to determine if CCL2/CCR2 antagonism will offer breakthrough therapy for patients with allergic asthma, IPF, or BOS, and to confirm the hypothesis that CCL2 polymorphism places patients at greater risk for these disorders. Microcirculation (2003) 10, 273-288. doi:10.1038/sj.mn.7800193

Record Date Created: 20030709

2/7/32 (Item 2 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
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14894594 22620887 PMID: 12735123  
Respiratory failure in elderly patients.  
Sevransky Jonathan E; Haponik Edward F

Division of Pulmonary and Critical Care Medicine, Johns Hopkins  
University School of Medicine, 5501 Hopkins Bayview Circle, Baltimore, MD  
21224-6801, USA. sevransj@welch.jhu.edu

Clinics in geriatric medicine (United States) Feb 2003, 19 (1)  
p205-24, ISSN 0749-0690 Journal Code: 8603766

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Elderly individuals comprise an increasing proportion of the population and represent a progressively expanding number of patients admitted to the ICU. Because of underlying pulmonary disease, loss of muscle mass, and other comorbid conditions, older persons are at increased risk of developing respiratory failure. Recognition of this vulnerability and the adoption of proactive measures to prevent decompensation requiring intrusive support are major priorities together with clear delineation of patients' wishes regarding the extent of support desired should clinical deterioration occur. Further, the development of coordinated approaches to identify patients at risk for respiratory failure and strategies to prevent the need for intubation, such as the use of NIV in appropriate patients, are crucial. As soon as endotracheal intubation and mechanical ventilation are implemented strategies that facilitate the liberation of elderly patients from the ventilator are especially important. The emphasis on a team approach, which characterizes geriatric medicine, is essential in coordinating the skills of multiple health care professionals in this setting. Respiratory failure can neither be effectively diagnosed nor managed in isolation. Integration with all other aspects of care is essential. Patient vulnerability to nosocomial complications and the "cascade effect" of these problems such as the effects of medications and invasive supportive procedures all impact on respiratory care of elderly patients. For example, prolonged mechanical ventilation may be required long after resolution of the underlying cause of respiratory failure because of unrecognized and untreated delirium or residual effects of small doses of sedative and/or analgesic agents or other medications in elderly patients with altered drug metabolism. The deleterious impact of the foreign and sometimes threatening ICU environment and/or sleep deprivation on the patient's course are too often overlooked because the physician focuses management on physiologic measurements, mechanical ventilator settings, and other technologic nuances of care [40]. Review of the literature suggests that the development of respiratory failure in patients with certain disease processes such as COPD, IPP, and ARDS in elderly patients may lead to worsened outcome but it appears that the disease process itself, rather than the age of the patient, is the major determinant of outcome. Additional studies suggest that other comorbid factors may be more important than age. Only when comorbid processes are taken into account should decisions be made about the efficacy of instituting mechanical ventilation. In addition, because outcome prediction appears to be more accurate for groups of patients rather than for individual patients a well-structured therapeutic trial of instituting mechanical ventilation, even if comorbidities are present, may be indicated in certain patients if appropriately informed patients wish to pursue this course. This approach requires careful and realistic definition of potential outcomes, focus on optimizing treatment of the reversible components of the illness, and continuous communication with the patient and family. Although many clinicians share a nihilistic view regarding the potential usefulness of mechanical ventilation in elderly patients few data warrant this negative prognostication and more outcome studies are needed to delineate the optimum application of this element of supportive care. As with other interventions individualization of the decision must take into account the patient's premorbid status, concomitant conditions, the nature of the precipitating illness and its prospects for improvement, and most important, patient preferences. In this determination pursuing the course most consistent with the patient's wishes is essential and it must be appreciated that caregivers' impressions regarding the vigor of support

desired by the patient are often erroneous. The SUPPORT investigators observed that clinicians often underestimated the degree of intervention desired by older patients assuming that less care would be desired [13]. Thus, as in other circumstances, effective communication and elicitation of patients' preferences regarding management options is crucial in the management of respiratory failure. The frequent discordance between patient preferences and the wishes of family members or other surrogate decision makers impose major clinical challenges and also mandates further investigation. (60 Refs.)

Record Date Created: 20030508

Record Date Completed: 20030523

2/7/33 (Item 3 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
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10435831 96242491 PMID: 8678788  
Cytokines in human lung fibrosis.  
Martinet Y; Menard O; Vaillant P; Vignaud J M; Martinet N  
INSERM U 14, Nancy-Vandoeuvre.  
Archives of toxicology. Supplement. Archiv fur Toxikologie. Supplement (GERMANY) 1996, 18 p127-39, ISSN 0171-9750 Journal Code: 7802567  
Document type: Journal Article; Review; Review, Tutorial  
Languages: ENGLISH  
Main Citation Owner: NLM  
Record type: Completed  
Fibrosis is a pathological process characterized by the replacement of normal tissue by mesenchymal cells and the extracellular matrix produced by these cells. The sequence of events leading to fibrosis of an organ involves the subsequent processes of injury with inflammation and disruption of the normal tissue architecture, followed by tissue repair with accumulation of mesenchymal cells in the area of derangement. The same sequence of events occurs in wound healing with normal granulation tissue and scar formation, but, while normal scar formation is very localized and transient, in contrast, in fibrosis, the repair process is exaggerated and usually widespread and can be chronic. Inflammatory cells (mainly mononuclear phagocytes), platelets, endothelial cells, and type II pneumocytes play a direct and indirect role in tissue injury and repair. The evaluation of three human fibrotic lung diseases, two diffuse [idiopathic pulmonary fibrosis (IPF), and the adult respiratory distress syndrome (ARDS)], and one focal (tumor stroma in lung cancer), has shown that several cytokines participate to the local injury and inflammatory reaction [interleukin-1 (IL-1), interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-alpha)], while other cytokines are involved in tissue repair and fibrosis [platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-beta (TGF-beta), and basic-fibroblast growth factor (b-FGF)]. A better understanding of the cytokines and cytokine networks involved in lung fibrosis leads to the possibility of new therapeutic approaches. (39 Refs.)

Record Date Created: 19960812

Record Date Completed: 19960812

2/7/34 (Item 4 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)  
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05532831 87211825 PMID: 3555299  
Lung inflammation: normal host defense or a complication of some diseases?  
Reynolds H Y  
Annual review of medicine (UNITED STATES) 1987, 38 p295-323, ISSN

0066-4219 Journal Code: 2985151R  
Document type: Journal Article; Review  
Languages: ENGLISH  
Main Citation Owner: NLM  
Record type: Completed

The inflammatory response in lung tissue is an important part of host defense that aids in removing microorganisms or particles that have reached the distal airways and alveolar surface. It augments the usual function of alveolar macrophages, immunoglobulins, and other defense mechanisms such as mucociliary clearance. However, excessive or poorly regulated inflammation can be destructive of tissue, thus contributing to many disease processes that can lead to fibrosis and impaired gas exchange. Several examples of diseases that feature inflammation as part of their pathophysiology have been selected for this review; i.e. asthma (especially examining the late-phase reaction that involves PMNs), chronic bronchitis (in which irritants and bacterial products may stimulate mucus secretion and inflammatory cells), interstitial lung diseases (IPF may disclose PMNs but sarcoidosis and hypersensitivity pneumonitis feature collections of activated lymphocytes), and acute lung injury leading to **adult respiratory** distress syndrome (PMNs and their breakdown products and enzymes incite local destruction of alveolar tissue). In preparation for these disease examples, a thorough review of the normal interactions between alveolar macrophages, various opsonins, complement and chemotactic factors, and the responsiveness of PMNs is given first. (124 Refs.)

Record Date Created: 19870610

Record Date Completed: 19870610

? s s2 and tgf?

34 S2  
81331 TGF?

S3 5 S2 AND TGF?

? rd s3

...completed examining records

S4 5 RD S3 (unique items)

? t s4/7/all

4/7/1 (Item 1 from file: 5)

DIALOG(R)File 5: Biosis Previews(R)

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14117115 BIOSIS NO.: 200300111144

Roles for insulin-like growth factor I and transforming growth factor-beta in fibrotic lung disease.

AUTHOR: Krein Peter M; Winston Brent W(a)

AUTHOR ADDRESS: (a)Departments of Medicine, Biochemistry and Molecular Biology, and Critical Care Medicine, Health Sciences Center, University of Calgary, 3330 Hospital Dr NW, Room 1843, Calgary, AB, T2N 4N1, Canada  
\*\*Canada E-Mail: bwinston@ucalgary.ca

JOURNAL: Chest 122 (6 Suppl.):p289S-293S December 2002 2002

MEDIUM: print

ISSN: 0012-3692

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

**ABSTRACT:** Idiopathic pulmonary fibrosis (IPF) is a lung disease that is characterized by epithelial cell damage and areas of denuded basement membrane resulting in inflammation, fibroblast proliferation, excessive extracellular matrix (ECM) deposition, and remodeling of alveolar gas exchange units. The progressive loss of lung gas exchange units in patients with IPF leads to respiratory failure and eventually to death. While the etiology of this disease is unknown, for many years studies suggested that chronic inflammation was the underlying factor that caused fibroproliferation and structural alterations of the lung. Recent data show that fibroproliferation and fibrosis can occur

independently of inflammation, suggesting that **IPF** is a disease caused by a mesenchymal, rather than an immune disorder. Mesenchymal growth factors, including transforming growth factor (**TGF**)-beta, insulin-like growth factor (**IGF**)-I, platelet-derived growth factor, connective tissue growth factor, fibroblast growth factors, and keratinocyte growth factors, as well as proinflammatory cytokines such as tumor necrosis factor-alpha and interleukin-1beta, have been shown to be exaggerated in several fibrotic lung disorders including **IPF**, **ARDS**, sarcoidosis, and bronchopulmonary dysplasia, as well as pulmonary manifestations of systemic diseases such as rheumatoid arthritis or progressive systemic sclerosis (scleroderma). We argue that inflammation is required to initiate growth factor production and repair of the damaged alveolar epithelial lining in fibrotic lung diseases and that exaggerated **TGF**-beta production may be responsible for the fibrotic response seen in diseases such as **IPF**. We recognize the potential role of several growth factors in the fibroproliferative process in the lung, and in this brief report we focus on the possible roles of the growth factors **IGF**-I and **TGF**-beta in cell migration, proliferation, and ECM synthesis in patients with **IPF**.

4/7/2 (Item 2 from file: 5)  
DIALOG(R)File 5: Biosis Previews(R)  
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11658919 BIOSIS NO.: 199800440650

Elevated transforming growth factor-alpha levels in bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome.

AUTHOR: Madtes David K(a); Rubenfeld Gordon; Klima Lawrence D; Milberg John A; Steinberg Kenneth P; Martin Thomas R; Raghu Ganesh; Hudson Leonard D; Clark Joan G

AUTHOR ADDRESS: (a) Fred Hutchinson Cancer Res. Center, 1124 Columbia St. M677, Seattle, WA 98104-2092\*\*USA

JOURNAL: American Journal of Respiratory and Critical Care Medicine 158 (2):p424-430 Aug., 1998

ISSN: 1073-449X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

**ABSTRACT:** The acute respiratory distress syndrome (**ARDS**) frequently results in a fibroproliferative response that precludes effective alveolar repair. Transforming growth factor-alpha (**TGF**-alpha), a potent epithelial and mesenchymal cell mitogen, may modulate the response to lung injury. In this study, we determined whether bronchoalveolar lavage fluid (BALF) concentrations of **TGF**-alpha are increased during the first 2 wk after the onset of **ARDS** and, if so, whether increased **TGF**-alpha levels in lavage fluid are associated with increased levels of procollagen peptide III (PCP III), a biological marker of fibroproliferation, and with increased fatality rates. We enrolled 74 consecutive patients with **ARDS** prospectively identified on admission to the intensive care unit of a tertiary care hospital, and 11 patients with chronic interstitial lung disease. Thirteen healthy volunteers served as control subjects. **TGF**-alpha-concentrations were measured in BALF recovered on Days 3, 7, and 14 after the onset of **ARDS** (total of 130 lavage samples). **TGF**-alpha was detected in the lavage fluid of 90% of patients with **ARDS** (67 of 74), and in 100% of patients with idiopathic pulmonary fibrosis (**IPF**) (10 of 10), but in none of 13 normal volunteers. At each day tested, the median lavage **TGF**-alpha level of patients with **ARDS** was significantly higher than that of normals. The overall fatality rate was 45% (33 of 74 patients). In a univariate analysis, the median **TGF**-alpha levels in nonsurvivors were 1.5-fold higher at Day 7 ( $p = 0.06$ ) and 1.8-fold higher at Day 14 ( $p = 0.048$ ). The fatality rate was 4 times

higher (CI 1.6, 17.5) for patients with both increased lavage TGF-alpha and PCP III concentrations at Day 7 than for patients with low TGF-alpha and PCP III values, indicating a synergistic relationship between TGF-alpha and PCP III. We conclude that increased levels of TGF-alpha in BALF are common in patients with ARDS and that lavage TGF-alpha is associated with a marker of the fibroproliferative response in sustained ARDS.

4/7/3 (Item 1 from file: 73)  
DIALOG(R)File 73:EMBASE  
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06519657 EMBASE No: 1996185319  
The role of cytokines in human lung fibrosis  
Vaillant P.; Menard O.; Vignaud J.-M.; Martinet N.; Martinet Y.  
Fed. Medico-Chirurgicale Pneumologie, CHU de Nancy-Brabois, Allée du  
Morvan, 54511 Vandoeuvre-les-Nancy France  
Monaldi Archives for Chest Disease ( MONALDI ARCH. CHEST DIS. ) (Italy)  
1996, 51/2 (145-152)  
CODEN: MACDE ISSN: 1122-0643  
DOCUMENT TYPE: Journal; Article  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Fibrosis is a disorder characterized by a qualitative and quantitative alteration of the deposition of extracellular matrix with accumulation of mesenchymal cells in replacement of normal tissue. The sequence of events leading to fibrosis of an organ involves the subsequent processes of injury with inflammation and disruption of the normal tissue architecture, followed by tissue repair with accumulation of mesenchymal cells in this area. A similar sequence of events occurs in wound healing with formation of normal, limited and transient granulation tissue, while in fibrosis, a maladaptive repair leads to an extensive, exaggerated process with functional impairment. Inflammatory cells (mainly mononuclear phagocytes), platelets, endothelial cells, and type II pneumocytes play a direct and indirect role in tissue injury and repair. The evaluation of several human fibrotic lung diseases, five diffuse (idiopathic pulmonary fibrosis (IPF); adult respiratory distress syndrome (ARDS); coal workers' pneumoconiosis (CWP); Hermansky-Pudlak syndrome (HPS); systemic sclerosis (SS)) and two focal (tumour stroma in lung cancer; and obliterative bronchiolitis (OB) after lung transplantation), has shown that several cytokines participate in the local injury and inflammatory reaction (interleukin-1 (IL-1), interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), and tumour necrosis factor-alpha (TNF-alpha)), while other cytokines are involved in tissue repair and fibrosis (platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-beta (TGF-beta), and basic-fibroblast growth factor (b-FGF)). A better understanding of the cytokines and cytokine networks involved in lung fibrosis leads to the possibility of new therapeutic approaches.

4/7/4 (Item 1 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)  
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15074455 22735455 PMID: 12851645  
Significant Involvement of CCL2 (MCP-1) in Inflammatory Disorders of the Lung.  
Rose C Edward; Sung Sung-Sang J; Fu Shu Man  
Division of Pulmonary and Critical Care Medicine and the Division of  
Rheumatology and Immunology, University of Virginia School of Medicine,  
Charlottesville, VA, USA.  
Microcirculation (New York, N.Y. - 1994) (United States) Jul 2003, 10

(3-4) p273-88, ISSN 1073-9688 Journal Code: 9434935

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: In Process

Mounting evidence suggests that CCL2 (MCP-1) and its hematopoietic cell receptor CC chemokine receptor 2 (CCR2) are involved in inflammatory disorders of the lung. In animal models of allergic asthma, idiopathic pulmonary fibrosis (IPF), and bronchiolitis obliterans syndrome (BOS), CCL2 expression and protein production are increased and the disease process is attenuated by CCL2 immunoneutralization. Mechanisms by which CCL2 may be acting include recruitment of regulatory and effector leukocytes; stimulation of histamine or leukotriene release from mast cells or basophils; induction of fibroblast production of transforming growth factor-beta (TGF-beta) and procollagen; and enhancement of Th2 polarization. Recently, polymorphism for CCL2 has been described with increased cytokine-induced release of CCL2 by monocytes and increased risk of allergic asthma. These studies identify potentially important roles for CCL2 in these lung inflammatory disorders. While CCL2 inhibition in patients with acute respiratory distress syndrome (ARDS) may be hazardous by interfering with defense against bacteremia, future studies are needed to determine if CCL2/CCR2 antagonism will offer breakthrough therapy for patients with allergic asthma, IPF, or BOS, and to confirm the hypothesis that CCL2 polymorphism places patients at greater risk for these disorders. Microcirculation (2003) 10, 273-288. doi:10.1038/sj.mn.7800193

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4/7/5 (Item 2 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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10435831 96242491 PMID: 8678788

Cytokines in human lung fibrosis.

Martinet Y; Menard O; Vaillant P; Vignaud J M; Martinet N

INSERM U 14, Nancy-Vandoeuvre.

Archives of toxicology. Supplement. Archiv fur Toxikologie. Supplement (GERMANY) 1996, 18 p127-39, ISSN 0171-9750 Journal Code: 7802567

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Fibrosis is a pathological process characterized by the replacement of normal tissue by mesenchymal cells and the extracellular matrix produced by these cells. The sequence of events leading to fibrosis of an organ involves the subsequent processes of injury with inflammation and disruption of the normal tissue architecture, followed by tissue repair with accumulation of mesenchymal cells in the area of derangement. The same sequence of events occurs in wound healing with normal granulation tissue and scar formation, but, while normal scar formation is very localized and transient, in contrast, in fibrosis, the repair process is exaggerated and usually widespread and can be chronic. Inflammatory cells (mainly mononuclear phagocytes), platelets, endothelial cells, and type II pneumocytes play a direct and indirect role in tissue injury and repair. The evaluation of three human fibrotic lung diseases, two diffuse [idiopathic pulmonary fibrosis (IPF), and the adult respiratory distress syndrome (ARDS)], and one focal (tumor stroma in lung cancer), has shown that several cytokines participate to the local injury and inflammatory reaction [interleukin-1 (IL-1), interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-alpha)], while other cytokines are involved in tissue repair and fibrosis [platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-beta (TGF-beta), and

basic-fibroblast growth factor (b-FGF)]. A better understanding of the cytokines and cytokine networks involved in lung fibrosis leads to the possibility of new therapeutic approaches. (39 Refs.)

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